PARASITOLOGY

(Founded by G. H. F. Nuttall, F.R.S.)

EDITED BY

D. KEILIN

IN CONJUNCTION WITH

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CONTENTS

Nos. 1, 2 (July 1948)

PAGE

Busvine, J. R. The 'head' and 'body' races of *Pediculus humanus* L. (With Plate I and 7 Figures in the Text) 1

Crofton, H. D. The ecology of immature phases of Trichostrongyle nematodes. I. The vertical distribution of infective larvae of *Trichostrongylus retortaeformis* in relation to their habitat. (With 5 Figures in the Text) 17

Crofton, H. D. The ecology of immature phases of Trichostrongyle nematodes. II. The effect of climatic factors on the availability of the infective larvae of *Trichostrongylus retortaeformis* to the host. (With 6 Figures in the Text) 26

Lawrence, R. F. Some new pilicolous mites from South African mammals. (With 8 Figures in the Text) 39

Satchell, G. H. The respiratory horns of *Psychoda* pupae (Diptera: Psychodidae). (With 27 Figures in the Text) 43

Arthur, D. R. On the egg of the tick, *Ixodes ricinus* L. (With 12 Figures in the Text) . . 53

MacLeod, John. The distribution and dynamics of ked populations, *Melophagus ovinus* Linn. (With 4 Figures in the Text) 61

Hansen, Eder L. and Anderson, Hamilton H. An essentially synthetic liquid medium for *Entamoeba histolytica* 69

Sproston, Nora G. On the genus *Dinobothrium* van Beneden (Cestoda), with a description of two new species from sharks, and a note on *Monorygma* sp. from the electric ray. (With 25 Figures in the Text) 73

Meyer, H. and Xavier de Oliveira, M. Cultivation of *Trypanosoma cruzi* in tissue cultures: a four-year study. (With Plates II and III) 91

Adler, S. and Feldman-Muehsam, B. A note on the genus *Hyalomma* Koch in Palestine. (With 8 Figures in the Text) 95

Tate, P. The technique for breeding pure-line cultures of the blow-fly (*Calliphora erythrocephala*). (With 3 Figures in the Text) 102

Rogers, W. P. The respiratory metabolism of parasitic nematodes. (With 2 Figures in the Text) 105

Tripathi, Yogendra R. Some new Myxosporidia from Plymouth with a proposed new classification of the Order. (With 6 Figures in the Text) 110

Buxton, P. A. Experiments with mice and fleas. I. The baby mouse. (With 2 Figures in the Text) 119

Bishop, Ann and Birkett, Betty. Drug-resistance in *Plasmodium gallinaceum*, and the persistence of paludrine-resistance after mosquito transmission 125

Feldman-Muehsam, B. On larvae and nymphs of some species of Palestinian *Hyalomma*. (With 12 Figures in the Text) 138

Sharif, M. The water relations of the larva of *Xenopsylla cheopis* (Siphonaptera) . . 148

Nos. 3, 4 (February 1949)

PAGE

Fastier, L. B. The effect of physical agents on hydatid scolex viability. (With 6 Figures in the Text) 157

Weiser, Jaroslav. Studies on some parasites of fishes. (With 2 Figures in the Text) . . 164

Milne, A. The ecology of the sheep tick, *Ixodes ricinus* L. Host relationships of the tick. Part 1. Review of previous work in Britain 167

Milne, A. The ecology of the sheep tick, *Ixodes ricinus* L. Host relationships of the tick. Part 2. Observations on hill and moorland grazings in northern England . . . 173

Wilson, S. G. *Trypanosoma uniforme–Trypanosoma vivax* infections in bovines and *Trypanosoma uniforme* infections in goats and sheep at Entebbe, Uganda. (With 22 Figures in the Text) 198

Gnanamuthu, C. P. A new copepod parasite, *Lernanthropus dussumieria* n.sp., from the gills of a Madras fish. (With 6 Figures in the Text) 209

van Cleave, Harley J. *Pseudoporrorchis teliger*, a new species of acanthocephala from Java. (With 5 Figures in the Text) 214

Dougherty, Ellsworth C. A list of the trichostrongylid lungworms (Phylum Nematoda) and a key to the six genera. (With 2 Figures in the Text) 218

Dougherty, Ellsworth C. The phylogeny of the nematode family Metastrongylidae Leiper, [1909]: a correlation of host and symbiote evolution. (With 27 Figures in the Text) . 222

Arthur, D. R. The scutum of the tick, *Ixodes ricinus* L. (With 9 Figures in the Text) . 235

Matthews, R. E. F. Reactions of *Cyphomandra betacea* to strains of potato virus *X* . . 241

Rogers, W. P. and Lazarus, Marian. The uptake of radioactive phosphorus from host tissues and fluids by nematode parasites. (With 5 Figures in the Text) 245

Gerichter, Ch. B. Studies on the nematodes parasitic in the lungs of Felidae in Palestine. (With 27 Figures in the Text) 251

Kirk, R. The differentiation and nomenclature of *Leishmania* 263

Crofton, H. D. The ecology of immature phases of trichostrongyle nematodes. III. Larval populations on hill pastures. (With 3 Figures in the Text) 274

Trim, A. R. The kinetics of the penetration of some representative anthelmintics and related compounds into *Ascaris lumbricoides* var. *suis*. (With 9 Figures in the Text) . . 281

Ganapati, P. N. and Tate, P. On the gregarine *Lankesteria culicis* (Ross), 1898, from the mosquito *Aëdes* (*Finlaya*) *geniculatus* (Olivier). (With 5 Figures in the Text) . . 291

Lewis, D. J. The distribution of Cimicidae (Hemiptera) in the Anglo-Egyptian Sudan. (With 4 Figures in the Text) 295

Chance, M. R. A. and Dirnhuber, P. The water-soluble vitamins of parasitic worms . . 300

Rogers, W. P. and Lazarus, Marian. Glycolysis and related phosphorus metabolism in parasitic nematodes. (With 6 Figures in the Text) 302

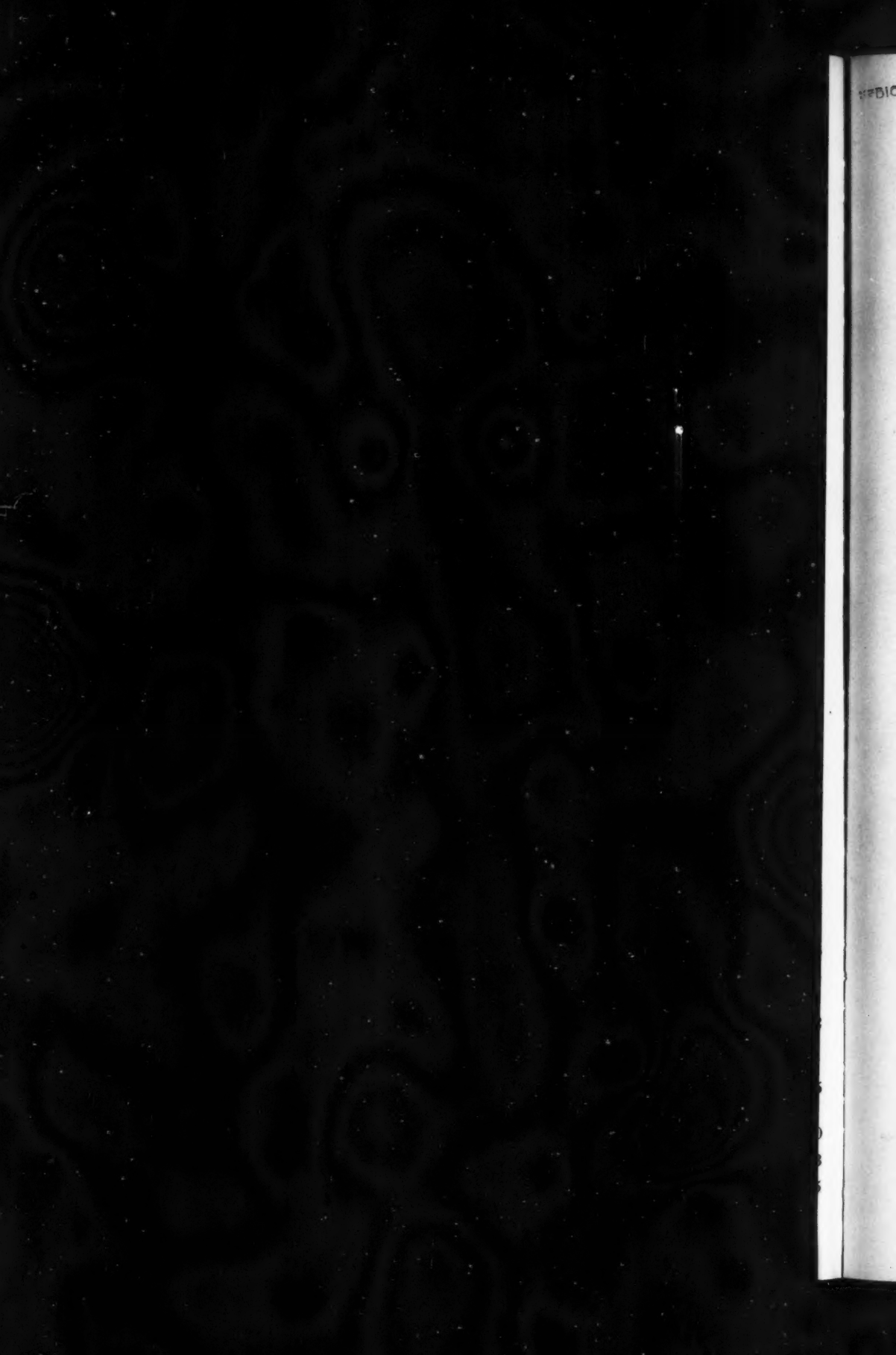
Jobling, B. Host-parasite relationship between the American Streblidae and the bats, with a new key to the American genera and a record of the Streblidae from Trinidad, British West Indies (Diptera). (With 3 Figures in the Text) 315

Markham, Roy and Smith, Kenneth M. Studies on the virus of turnip yellow mosaic. (With Plates IV–VII and 4 Figures in the Text) 330

Index of Authors 343

Index of Subjects 345

PAGE
157
164
167
173
198
209
214
218
222
235
241
245
251
263
274
281
291
295
300
302
315
330
343
345



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VOLUME 39, NOS. 1, 2 JULY 1948

THE 'HEAD' AND 'BODY' RACES OF *PEDICULUS HUMANUS* L.

BY J. R. BUSVINE, *Entomology Department, London School of Hygiene and Tropical Medicine*

(With Plate I and 7 Figures in the Text)

CONTENTS

	PAGE
I. Theoretical section	1
A. Introduction	1
B. Summary of the evidence	1
(1) Morphological	1
(2) Influence of environment on morphological differences	2
(3) Results of crossing *capitis* and *corporis* strains	2
(4) Biology of the two strains	2
C. Conclusions	3
II. Experimental section	3
A. Methods	3
(1) Sources of lice	3
(2) Methods of rearing in the laboratory	3
(3) Methods of fixing and preserving specimens	3
(4) Methods of measurement	4
B. Results of measurements	4
(1) Samples of 'wild' populations	4
(2) Measurements of strains bred in captivity	6
(3) Measurements of 'hybrids'	10
(4) Biological observations on 'captive' strains	10
(5) Discussion of experimental section	14
Summary	16
References	16

I. THEORETICAL SECTION

A. INTRODUCTION

In his 'Monograph of the Sucking Lice', Ferris (1935) reviews the evidence relating to the status of *Pediculus* '*capitis*' and *P.* '*corporis*'. He points out that some of this evidence is conflicting, so that it is difficult to reach a final decision. He says: 'we have gone as far as we can on the basis of the conventional methods of the systematist. It remains for extensive experimental work to carry further the investigation of the problem which has been set.' In recent years, other aspects of louse biology have received considerable attention; but with this particular problem, little progress has been made since the fundamental studies of Bacot, Nuttall, Keilin, Hase and Sikora at the time of the first World War. This paper is a contribution towards the deficit.

B. SUMMARY OF THE EVIDENCE

(1) *Morphological*

There are some obvious differences between typical specimens of lice taken from the head and from the body; and, given a few such thoroughly typical specimens, almost any systematist would be inclined to recognize them as separate species. If larger, more representative, samples are examined, however, the following conclusions will be drawn:

(*a*) *Size and proportion*

The average size of head lice is less than the average size of body lice, whether gross body length or the size of any particular part is measured. However, there is considerable variation and overlap, so that it is possible to find small body lice which are exceeded by the largest head lice, and the same is true of measurements of parts of the body.

A prominent example is provided by the antennae of the two forms which show considerable differences in proportions, the ratio of their lengths being about 5 to 4. On the other hand, there is little or no difference in thickness so that those of the body louse appear noticeably more slender.

This difference has long been recognized and is noted by Nuttall. However, Ferris observes that 'gradations between the two extremes appear in material which may reasonably be used in this comparison'.

(*b*) *Shape*

The lateral indentations between segments of the abdomen are more pronounced in typical head lice than in body lice. This is, however, too vague and unreliable a character for the identification of individual lice.

According to Freund (1925) there are differences in the form and chaetotaxy of the genitalia of the two races; but Ferris discusses these in detail and dismisses them as trivial and inconstant.

(*c*) *Colour*

Head lice are generally darker than body lice; but this is a very variable character, largely depending on the environment (background coloration).

(*d*) *Musculature*

An interesting difference between the two forms was pointed out by Landois (1865), but seems to have been overlooked by most authors except Sikora. According to Landois, the body race has two sets of longitudinal muscles on the ventral body wall of the abdominal segments 4 and 5. (See Plate I.) In the head louse only the muscles on the 5th segment are present. This would prove an important categorical distinction, but unfortunately Sikora observes (1919) that it is not absolutely consistent. She attributes this to interbreeding between the two races, remarking that the pure body strain is comparatively rare.

(*e*) *Dimensions of the egg*

In a recent study, Alpatov, Nastukova & Chartulari (1945) measured six dimensions of the louse egg and took the position of the egg cement as a seventh character. From these measurements, averages with standard errors were calculated and are given in tabular form. On the basis of these figures, it seems clear that the eggs of the head and body forms of lice are recognizably distinct (judged by means) in natural populations.

(2) *Influence of environment on morphological differences*

Nuttall believed that many of the characters of the louse, and in particular those distinguishing the head and body forms, were dependent on the environment. If, however, a critical examination is made of the data on which he bases his conclusions, some of it will be found imprecise or otherwise unsatisfactory. Thus:

(*a*) *Concerning pigmentation of lice*

Contrary to Hindle's (1917) conclusions, Nuttall (1919*a*) states that 'pigmentation in *Pediculus* is not an hereditary character; its presence depends entirely upon the nature of the background upon which the insect lives and is a character that may be acquired in a couple of days'. This statement is too categorical. I have shown recently (1946) that although the pigmentation itself is not inherited, the ability to react to a background is hereditable, so that there is some genetic mechanism involved.

(*b*) *Transformation of 'capitis' to 'corporis'*

Nuttall states (1919*b*) that 'typical *capitis* lose all their distinctive morphological characters when raised experimentally on man under conditions which are favourable for the propagation of *corporis*, and they acquire all the morphological characters of *corporis* after four or more generations'. This is based on the following evidence:

(i) Sikora (1917) bred head lice for several generations on her arm. She stated that the head louse appeared to increase in size and become more like the body louse up to the 5th generation. Later, however, she recanted and affirms (1919) that head lice up to the 11th generation were quite recognizably distinct. This second paper is not mentioned by Nuttall or by Ferris.

(ii) Howlett (1918), in a brief paper, describes an experiment in which head lice (from an unstated source) were liberated on the body of his assistant. They tended to return to the head, but this reaction became less marked in F_1 and F_2 generations, while characters typical of the *corporis* form appeared.

(iii) Keilin & Nuttall (1919) record that they received from Bacot specimens of a *corporis* strain that had been reared by him for 2 years in the laboratory. These were found to be intermediate in character between typical *capitis* and typical *corporis*. A year later a further batch of the same strain was received, and the specimens were now found to be entirely of the *corporis* type.

The main criticism of this data is that it is mainly qualitative; the only figures given are a few measurements made by Sikora. Howlett and Keilin and Nuttall confine themselves to the bald statement that the typical *corporis* characters were observed.

Recently, however, a Russian paper has given support to this apparent mutability of the *capitis* strain. Alpatov *et al.* (1945) found that the eggs of a strain of head lice became similar in dimensions to those of body lice when reared in the laboratory. The change was apparently accomplished in the first generation and continued for several more.

(3) *Results of crossing 'capitis' and 'corporis' strains*

Bacot (1917) crossed strains of *capitis* and *corporis* and maintained the progeny for three generations. The cross-matings were easily accomplished and the 'hybrids' were vigorous. Keilin & Nuttall (1919), however, in examining Bacot's material, found an unusually high preponderance of hermaphrodites as compared with natural populations of either strain.

(4) *Biology of the two strains*

Bacot (1917) made numerous observations on the length of life, egg production and fertility of the head and body forms and gives his results in detail. His data are rather difficult to summarize quantitatively, but they clearly show a considerable difference between the two strains. The body louse, he says, 'is a larger, more robust and less active insect than *P. capitis*—the females having a relatively greater egg-carrying capacity than those of the head louse. The eggs are larger and the number

laid (under the conditions of the experiments) is greater, while the habits associated with egg-laying differ, although placing the females of *humanus* under conditions applicable to *capitis* or vice versa may induce a considerable degree of uniformity.'

C. CONCLUSIONS

As Ferris suggests, there are certain inconsistencies in the available evidence regarding the status of the two forms of *P. humanus*. To illustrate this concisely, two columns can be drawn contrasting (1) the facts suggesting that the two forms are merely extremes of a single variable species, with (2) facts supporting the existence of two distinct varieties.

Supporting uniformity	Supporting heterogeneity
Morphological	
(*a*) Both types are variable and all degrees of every character can be found. (*b*) Nuttall, and certain other authors, believed that such morphological differences as exist are dependent on environment, and that the *capitis* form reared on the body becomes transformed into the *corporis* type.	(*a*) Typical specimens differ obviously in several particulars, mainly of size and proportion. (*b*) There is very little *quantitative* evidence for this. Also there is disagreement on the time required for the change (2–3 years with Bacot's material; four generations according to Howlett; one generation according to Alpatov and colleagues).
Hybridization	
The two forms can readily be crossed and the progeny are vigorous and fertile.	There was an unusually high incidence of hermaphrodites in the progeny of Bacot's cross-matings, which is evidence of genetic incompatability.
Biology	
The general outlines of the biology are similar.	There are obvious differences in length of life, oviposition rate, etc.

II. EXPERIMENTAL SECTION

A. METHODS

(1) *Sources of lice*

Specimens of lice were taken from naturally infested individuals in London at different times. The head lice were usually taken from women and the body lice from men. Whenever possible, it was ascertained that the subject had only one type of infestation; that is, not head *and* body lice together. Such specimens will be referred to, for convenience, as 'wild' lice.

In addition, cultures of lice were maintained in breeding capsules with regular or continuous opportunities for feeding on human blood; these will be referred to as 'captive' strains. In particular, four strains were cultured for comparatively long periods as indicated in the diagram of Fig. 1. B 12 is a strain of body lice which has been maintained in the Department of Entomology of the London School of Hygiene and Tropical Medicine for about 10 years. In 1940 and 1941 it was reinforced by additional wild body lice. The lice in this culture are given opportunity to feed for about 12 hr. daily. B 24 was a strain taken from B 12 and worn day and night to allow opportunity to feed 24 hr. per day.

H 24 was a strain of head lice started from an infested person in February 1943 and maintained for over 2 years in captivity with opportunity to feed 24 hr. per day. H 12 was split off from H 24 and worn for 12 hr. daily. In July 1945, H 24 and H 12 were unfortunately lost and B 24 was discontinued 2 months later.

(2) *Methods of rearing in the laboratory*

The lice were reared in captivity in small feeding boxes or tins as described by Buxton (1940), with, however, one small difference. Head lice, as pointed out by Rocha-Lima & Sikora (1925) do not readily feed through gauze and they are also much more sensitive to starvation than body lice. Breeding boxes kept under the sock sometimes tend to slip about when walking so that opportunities to feed may be lost and head lice sometimes become weakened in such circumstances. Accordingly the head lice were kept in small flat tins bound to the ankles with elastic bandage. For the purpose of comparison the body lice were reared in exactly the same way.

(3) *Methods of fixing and preserving specimens*

It has often been remarked that lice are unsatisfactory subjects for measurement owing to their lack of a rigid cuticle and to their habit of taking large meals of blood at intervals, thereby distending the body. Preliminary tests showed that the method of preservation affects the final size; for example, half a batch of lice preserved in 50 % alcohol were from 2 to 10 % larger in several dimensions than the other half of the batch which was preserved in 100 % alcohol. To maintain uniformity, all lice were killed and preserved by plunging them into 70 % alcohol. All the various batches of specimens were kept in little plugged vials in a large bottle of this fluid.

The variation due to feeding was counteracted as far as possible by allowing the insects (whether wild or from laboratory strains) opportunity to feed on the skin for about an hour before they were killed and preserved. The majority of the specimens were, therefore, fully fed.

(4) *Methods of measurement*

For measurements of different parts of the body, lice were laid on their backs on a glass slide, covered with a portion of another slide and surrounded with 70 % alcohol run in from a pipette. Measurements were made by a micrometer eyepiece in an ordinary compound microscope. The total body length was observed through a 50 mm. objective (when the divisions of the scale recorded $\frac{1}{10}$ mm.) and the other dimensions were measured with a 16 mm. objective (when the divisions recorded $\frac{1}{60}$ mm.). As a rule, ten to twelve males and the same number of females were measured from each batch of lice and the means and standard deviations calculated from them.

B. Results of measurements

(1) *Samples of 'wild' populations*

(*a*) *Linear measurements of adult lice*

The principal dimensions which have been used to discriminate between *capitis* and *corporis* are the following: total body length, length of antennae, length of the 3rd segment of the antenna, width of the thumb-like process of the anterior leg of the male and the total length of the 3rd tibia. In actual practice, there is difficulty in measuring legs in whole specimens because they are usually curved under the body; even if they are partially straightened, there is likely to be a big error of parallax. Similar considerations apply to a smaller extent with regard to the antennae. I therefore chose to measure the

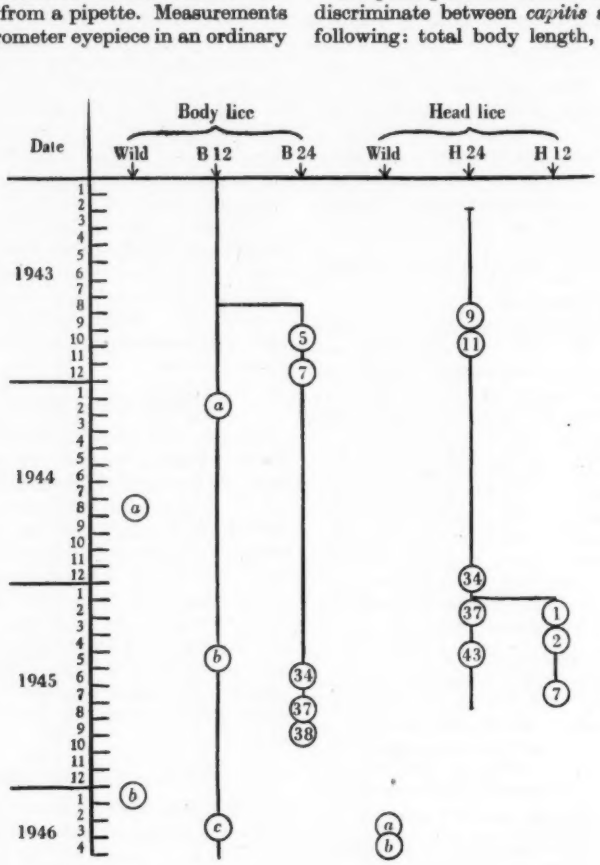

Fig. 1. Diagram to illustrate the origin of samples of lice from strains reared in captivity. H = head strain; B = body strain; 12 and 24 = number of hours per day they were allowed access to food. The figures in the circles give the number of generations under the stated conditions; the letters refer to other samples of lice, of unknown generations.

following dimensions: (*a*) total body length, (*b*) length of head, (*c*) width of head, (*d*) length of distal four segments of antenna, (*e*) length of 3rd antennal segment, (*f*) width of 3rd antennal segment. The exact location of these measurements are shown in Fig. 2. Tables 1 and 2 give the means of these dimensions for two 'wild' samples of body lice and two 'wild' samples of head lice. In all cases the means for body lice are significantly larger than those for head lice except for the *width* of the 3rd antennal segment, which is about the same in both forms. These facts are illustrated graphically in Fig. 3 in which it will be seen that for the first five dimensions, the means are distinct though there is a considerable overlap. (Some measurements of captive lice with the same means were included in these graphs.)

the 3rd antennal segment is least satisfactory; this may result from a greater error due to its smaller size relative to the scale divisions. From the practical point of view the first three dimensions are considerably more convenient to measure than the last three. When a whole louse is laid on a slide, the antennae may lie at an angle to the horizontal and cannot readily be levelled with the cover glass owing to the thickness of the body. Measurements of antennae at an angle have to be avoided owing to the error of parallax. In addition to this, the small dimensions of the antennal joints require a high power objective for accuracy.

Under the circumstances, it was considered satisfactory to use the first three dimensions in subsequent measurements.

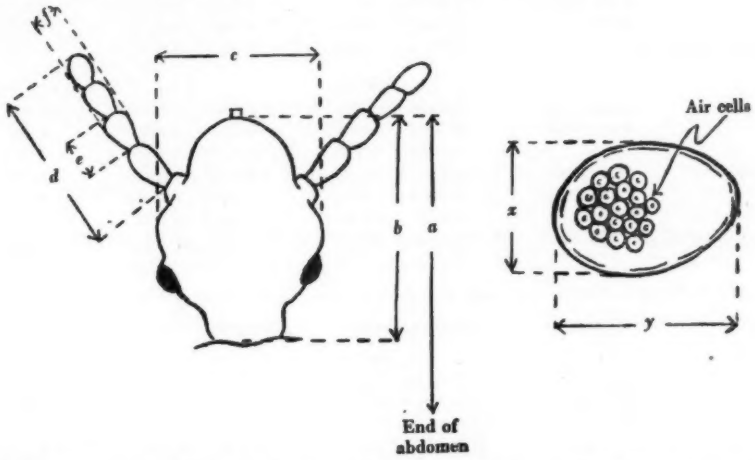

Fig. 2. Positions of measurements made to discriminate between different strains of lice. *a*, total length; *b*, head length; *c*, head width; *d*, antenna length (distal 4 segments); *e*, length of 3rd antennal segment; *f*, width of 3rd antennal segment. On right, the egg cap; *y*, length and *x*, width.

To form an impression as to the value of the various measurements in distinguishing between samples of head and body lice, the ratios of the differences between the means to their standard errors were calculated as follows:

Measurements on 'wild' lice	Difference / Standard error of difference		
	Males	Females	Average
(*a*) Head length	10·0	14·8	12·4
(*b*) Head width	8·5	15·1	11·8
(*c*) Body length	9·3	15·1	12·2
(*d*) Antenna length	11·7	15·6	13·1
(*e*) 3rd antennal segment	6·6	11·1	8·8

There seems to be no outstanding difference in the relative merits of the various measurements for discriminating between the two forms. The length of

(*b*) *Observations of abdominal musculature in females*

It is unfortunately somewhat difficult to determine the status of the abdominal muscles in the female lice preserved in 70% alcohol. The muscles can be seen in a proportion of lice from the outside (as described by Sikora). But in other specimens, less well preserved, they are difficult to examine without very careful dissection. However, the following results were obtained by direct observation: of sixty-six wild body lice, the musculature could be distinguished in forty-four specimens. Of these, ten had muscles in segment 5 only, forty-five had muscle rows in segments 4 and 5 (and one specimen had in addition a pair of muscles in segment 3!—see Plate I). Of forty-one wild head lice, the muscles could be observed in thirty-three specimens, all of which had muscles in segment 5 only.

In the samples examined, then, the muscles were confined to segment 5 in the head lice but were *usually* present in segment 4 as well in body lice.

(2) *Measurements of strains bred in captivity*

(*a*) *Linear measurements*

Samples of the four captive strains were preserved at various times, when there were sufficient adults available, as indicated in Fig. 1. Normally a population of two or three dozen adults was achieved in each generation, but sometimes there were less or the population was nearly unisexual. (Unbalanced sex ratios are common in small louse cultures.) It will be admitted that the disposition of these samples leaves much to be desired for making exact comparisons; but the work was done in the intervals of more urgent war-time tasks connected with insecticides.

Table 3 gives mean figures for the head length and width and the total body length of samples preserved from the various strains at different times. A careful examination of this table reveals no consistent trend in any dimension of either of the head or body strains. The various samples do not show differences greater than would be expected in normal sampling of a population.

Table 1. *Mean dimensions* (*in mm. with standard errors*) *of lice from natural infestations*

Strain	Date of sample	Head length	Head width	Total length
		Males		
Body lice	Aug. 1944	0·5037 ± 0·0046	0·4016 ± 0·0043	3·322 ± 0·047
	Jan. 1946	0·5222 ± 0·0119	0·4000 ± 0·0043	3·150 ± 0·084
	Both	0·5112 ± 0·0057	0·4012 ± 0·0030	3·253 ± 0·047
Head lice	May 1946	0·4667 ± 0·0033	0·3542 ± 0·0033	2·675 ± 0·052
	June 1946	0·4573 ± 0·0055	0·3722 ± 0·0055	2·822 ± 0·070
	Both	0·4602 ± 0·0045	0·3667 ± 0·0050	2·777 ± 0·054
Combined difference		0·0510 ± 0·0051	0·0345 ± 0·0041	0·476 ± 0·051
		Females		
Body lice	Aug. 1944	0·5406 ± 0·0016	0·4346 ± 0·0062	3·808 ± 0·082
	Jan. 1946	0·5622 ± 0·0095	0·4350 ± 0·0040	3·737 ± 0·042
	Both	0·5515 ± 0·0022	0·4350 ± 0·0037	3·774 ± 0·046
Head lice	May 1946	0·4955 ± 0·0035	0·3850 ± 0·0030	3·183 ± 0·033
	June 1946	0·4959 ± 0·0080	0·3909 ± 0·0035	3·158 ± 0·104
	Both	0·4957 ± 0·0040	0·3882 ± 0·0023	3·171 ± 0·034
Combined difference		0·0558 ± 0·0032	0·0468 ± 0·0031	0·603 ± 0·040

Table 2. *Mean dimensions* (*in mm. with standard errors*) *of antennae of lice from natural infestations*

Strain	Date of sample	Antenna length	3rd joint length	3rd joint width
		Males		
Body lice	Aug. 1944	0·3750 ± 0·0095	0·0990 ± 0·0025	0·0573 ± 0·0019
	Jan. 1946	0·3778 ± 0·0075	0·0932 ± 0·0026	0·0570 ± 0·0013
	Both	0·3762 ± 0·0060	0·0965 ± 0·0019	0·0572 ± 0·0012
Head lice	May 1946	0·3083 ± 0·0047	0·0772 ± 0·0040	0·0542 ± 0·0023
	June 1946	0·3188 ± 0·0059	0·0855 ± 0·0027	0·0625 ± 0·0015
	Both	0·3153 ± 0·0043	0·0827 ± 0·0023	0·0597 ± 0·0017
Combined difference		0·0609 ± 0·0052	0·0138 ± 0·0021	−0·0025 ± 0·0015
		Females		
Body lice	Aug. 1944	0·3903 ± 0·0060	0·1013 ± 0·0025	0·0612 ± 0·0012
	Jan. 1946	0·3820 ± 0·0067	0·0980 ± 0·0027	0·0597 ± 0·0010
	Both	0·3862 ± 0·0057	0·0997 ± 0·0019	0·0605 ± 0·0010
Head lice	May 1946	0·3092 ± 0·0035	0·0795 ± 0·0020	0·0613 ± 0·0023
	June 1946	0·3222 ± 0·0043	0·0840 ± 0·0012	0·0653 ± 0·0008
	Both	0·3160 ± 0·0030	0·0819 ± 0·0012	0·0633 ± 0·0012
Combined difference		0·0702 ± 0·0045	0·0178 ± 0·0016	−0·0028 ± 0·0011

Accordingly the figures for the various strains have been lumped together and the overall means are given in Table 4. This table also shows the standard errors for differences in class means. It will be seen that the difference between the head and body forms are in all cases highly significant.

The difference between lice fed 12 hr. and lice fed 24 hr. per day is also significant for most dimensions. The lice worn 24 hr. per day had a tendency to be smaller than those worn only 12 hr. per day and left

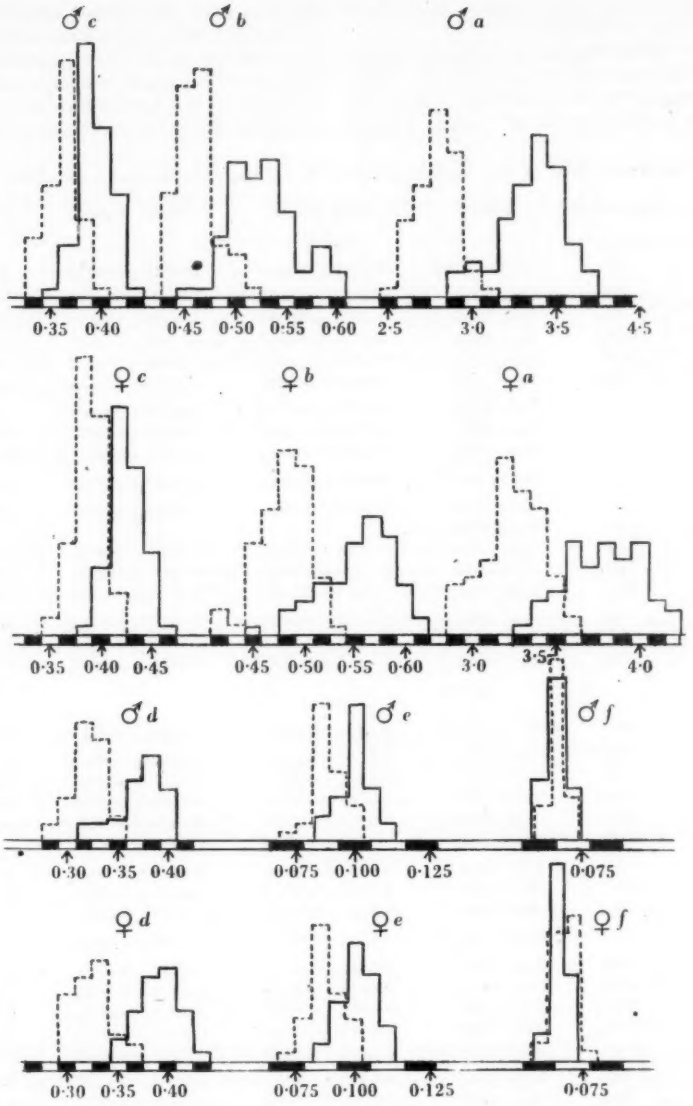

Fig. 3. Histograms showing distribution of sizes of various dimensions of head and body strains of lice. a = total body length; b = length of head; c = width of head; d = length of distal 4 segments of antenna; e = length of 3rd antennal joint; f = width of 3rd antennal joint. Solid line, body lice; dotted line, head lice.

at room temperature for the remaining period. It seemed likely that this might have been a temperature effect, and accordingly an experiment was designed to check this.

A batch of 1st-stage lice, fed once, was divided into two halves and one-half was fed 12 hr. per day and kept at room temperature for the other period, as usual. The other half was fed 12 hr. per day and kept on the body for the remaining 12 hr., but without opportunity to feed. Thus, both were fed 12 hr. a day, but one lot was, on the average, kept warmer than the other. The dimensions of the adults of these two half batches are given in Table 5. It will be seen that, in most dimensions, the lice reared under warm

conditions were significantly smaller than those reared under cooler conditions.

Alpatov *et al.* (1945) suggest as a possible reason for the smaller average size of head lice, that their environment may be warmer than that of body lice. They give no measurements of human hair or Bourne, 1946) to compare air temperatures at the roots of the hair and between underwear and skin of the same individuals. The recording instrument was kept at a distance of 3 mm. from the skin by a glass collar in all the measurements. The results are recorded in Table 14 and it will be observed that the

Table 3. *Mean dimensions (in mm. with standard errors) of lice from strains maintained in breeding tins*

Strain	Generation	Date	Head length	Head width	Total length
			Males		
B wild	—	1944 and 1946	0·5112 ± 0·0057	0·4012 ± 0·0030	3·253 ± 0·059
B 24	5th	Oct. 1943	0·5097 ± 0·0098	0·3983 ± 0·0077	3·383 ± 0·093
	7th	Dec. 1943	0·5763 ± 0·0063	0·4028 ± 0·0035	3·367 ± 0·025
	34th	May 1945	0·5138 ± 0·0055	0·3900 ± 0·0025	3·475 ± 0·023
	37th	Aug. 1945	0·5208 ± 0·0058	0·3833 ± 0·0023	3·425 ± 0·027
	38th	Sept. 1945	0·5308 ± 0·0087	0·3820 ± 0·0040	3·250 ± 0·040
B 12	—	Feb. 1944	0·5072 ± 0·0063	0·4116 ± 0·0057	3·214 ± 0·075
	—	May 1945	0·5305 ± 0·0082	0·4152 ± 0·0027	3·582 ± 0·044
	—	Mar. 1946	0·5263 ± 0·0082	0·4075 ± 0·0053	3·225 ± 0·030
H wild	—	1946*a* and *b*	0·4602 ± 0·0045	0·3667 ± 0·0050	2·777 ± 0·054
H 24	9th	Aug. 1943	0·4555 ± 0·0068	0·3555 ± 0·0095	2·767 ± 0·080
	11th	Sept. 1943	—	—	—
	34th	Dec. 1944	0·4528 ± 0·0035	0·3667 ± 0·0020	2·725 ± 0·023
	37th	Feb. 1945	0·4575 ± 0·0070	0·3607 ± 0·0050	2·817 ± 0·037
	43rd	May 1945	0·4627 ± 0·0060	0·3612 ± 0·0032	2·825 ± 0·025
H 12	1st	Feb. 1945	0·4440 ± 0·0060	0·3667 ± 0·0032	2·867 ± 0·034
	2nd	Mar. 1945	0·4458 ± 0·0057	0·3653 ± 0·0025	2·705 ± 0·026
	7th	July 1945	0·4458 ± 0·0050	0·3667 ± 0·0032	2·933 ± 0·032
			Females		
B wild	—	1944 and 1946	0·5515 ± 0·0022	0·4350 ± 0·0037	3·774 ± 0·046
B 24	5th	Oct. 1943	0·5167 ± 0·0012	0·4195 ± 0·0068	3·575 ± 0·096
	7th	Dec. 1943	0·5667 ± 0·0107	0·4167 ± 0·0068	3·575 ± 0·086
	34th	May 1945	0·5667 ± 0·0053	0·4208 ± 0·0028	3·950 ± 0·036
	37th	Aug. 1945	0·5800 ± 0·0105	0·4250 ± 0·0037	3·933 ± 0·048
	38th	Sept. 1945	0·5695 ± 0·0045	0·4195 ± 0·0028	3·675 ± 0·047
B 12	—	Feb. 1944	0·5167 ± 0·0060	0·4398 ± 0·0100	3·788 ± 0·103
	—	May 1945	0·5680 ± 0·0093	0·4445 ± 0·0083	3·983 ± 0·063
	—	Mar. 1946	0·5737 ± 0·0042	0·4375 ± 0·0028	3·783 ± 0·038
H wild	—	1946*a* and *b*	0·4957 ± 0·0040	0·3882 ± 0·0023	3·171 ± 0·034
H 24	9th	Aug. 1943	0·4682 ± 0·0053	0·3792 ± 0·0042	3·217 ± 0·040
	11th	Sept. 1943	0·4575 ± 0·0082	0·3875 ± 0·0045	3·283 ± 0·045
	34th	Dec. 1944	0·4682 ± 0·0082	0·3908 ± 0·0040	3·358 ± 0·043
	37th	Feb. 1945	0·4847 ± 0·0038	0·3987 ± 0·0038	3·367 ± 0·046
	43rd	May 1945	0·4875 ± 0·0050	0·3820 ± 0·0043	3·125 ± 0·041
H 12	1st	Feb. 1945	0·4847 ± 0·0032	0·3985 ± 0·0005	3·464 ± 0·021
	2nd	Mar. 1945	0·4750 ± 0·0028	0·3930 ± 0·0025	3·292 ± 0·034
	7th	July 1945	0·4958 ± 0·0080	0·4030 ± 0·0027	3·608 ± 0·017

B = body strain; H = head strain; 12 and 24 = number of hours allowed to feed per day.

clothing temperatures, nor do I know of any body of data in which this comparison can be made. (Though Marsh & Buxton (1937) and Mellanby (1932) record measurements of the body louse environment for sedentary people indoors at various temperatures.) Accordingly, some tentative experiments were made with a sensitive electrical thermometer (described by hair was usually *cooler* than the body environment. These data, admittedly limited, certainly give no support to the opposite suggestion.

(*b*) *Weights of adult lice*

A number of comparisons of weights of certain strains were made. For each sample, about ten live

lice of each sex from each strain were weighed individually on a torsion balance to the nearest 0·05 mg. The means and standard errors calculated from these weighings are given in Table 6. In general they confirm the results of the dimensional measurements, in that the head louse strain is consistently lower than the body strain and there are no obvious trends tending towards fusion. The difference between the two strains is proportionally greater, as would be expected, since the weight will be roughly proportional to the volume and hence to the cube of any linear dimension.

Table 4. *Mean dimensions (in mm. with standard errors) of all lice in each strain reared in breeding tins*

Strain	Head length	Head width	Total length
	Males		
B12	0·5237 ± 0·0043	0·4117 ± 0·0025	3·353 ± 0·041
B24	0·5303 ± 0·0140	0·3907 ± 0·0020	3·380 ± 0·070
H12	0·4452 ± 0·0029	0·3662 ± 0·0027	2·836 ± 0·070
H24	0·4573 ± 0·0029	0·3618 ± 0·0022	2·786 ± 0·054
Differences B–H	0·1515	0·0744	1·111
12–24	−0·0187	0·0254	0·023
Interaction	0·0055	0·0166	0·071
Standard error	0·0152	0·0047	0·120
	Females		
B12	0·5600 ± 0·0058	0·4402 ± 0·0030	3·859 ± 0·041
B24	0·5555 ± 0·0052	0·4203 ± 0·0022	3·811 ± 0·036
H12	0·4852 ± 0·0032	0·3990 ± 0·0002	3·454 ± 0·027
H24	0·4737 ± 0·0030	0·3875 ± 0·0020	3·270 ± 0·022
Differences B–H	0·1566	0·0740	0·946
12–24	0·0160	0·0314	0·232
Interaction	0·0070	0·0084	0·136
Standard error	0·0090	0·0046	0·064

Table 5. *Mean dimensions (in mm. with standard errors) of a single brood of lice, halved and reared under slightly different conditions*

Strain	Conditions when not feeding	Head length	Head width	Total length
		Males		
B12	Cool	0·5595 ± 0·0057	0·4167 ± 0·0025	3·400 ± 0·051
	Warm	0·5320 ± 0·0045	0·4000 ± 0·0045	3·475 ± 0·029
Difference ± S.E.		0·0275 ± 0·0051	0·0167 ± 0·0036	−0·075 ± 0·041
		Females		
B12	Cool	0·5917 ± 0·0038	0·4528 ± 0·0020	3·875 ± 0·038
	Warm	0·5695 ± 0·0055	0·4308 ± 0·0028	3·892 ± 0·051
Difference ± S.E.		0·0222 ± 0·0047	0·0220 ± 0·0024	−0·017 ± 0·045

Cool = room temperature when not feeding; warm = skin temperature when not feeding.

(c) *Observations on the eggs*

An examination of eggs of the two strains was made and it was concluded that the most rigid portion, and one which allowed of precise measurement, was the egg cap or operculum. By brushing clusters of hatched eggs, the opercula were easily detached and could be placed on a glass slide for measurements with the micrometer eyepiece. In addition to measurements of the long and short diameters of the oval egg cap, a note was made of the number of air cells in it. This number was found to vary considerably in different eggs laid by the same female, but the average numbers were greater with body louse eggs than those of head lice.

Figs. 4 and 5 show graphically the distribution of the dimensions of the opercula and the numbers of air cells in them for a large sample of eggs from 'captive' head and body lice. In Fig. 5 the distribution of air cell numbers of fifty eggs from a natural infestation is also shown. These graphs, like those of Fig. 3, show distinct means with considerable overlaps.

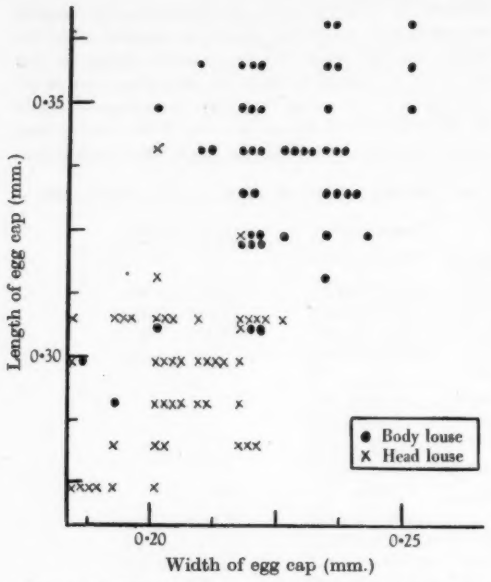

Fig. 4. Distribution of opercula measurements of eggs of head lice and body lice.

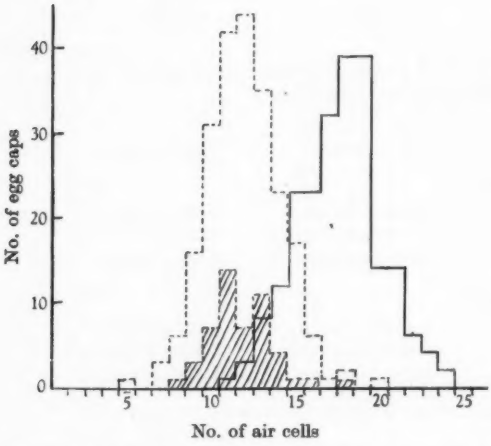

Fig. 5. Distribution of numbers of air cells in opercula of eggs of body lice (solid line) and head lice (broken line). The shaded portion gives the distribution of numbers of cells in fifty eggs from naturally infested hair.

(3) *Measurements of 'hybrids'*

(*a*) *Linear measurements*

A virgin female body louse of the strain B 24 (33rd generation) was mated with a male head louse of strain H 24 (33rd generation). The progeny were reared for several generations and samples were preserved of the F_1, F_2 and F_3 sibs. Some mean linear dimensions of the F_1 and F_3 adults are given in Table 7 and contrasted with similar measurements for all the B 24 and H 24 samples. It will be observed that the F_1 generation is intermediate in size between the body form and the head form, but the F_3 generation is indistinguishable from the body louse in size. The culture was worn for 24 hr. per day.

(*b*) *Weights of adults*

The mean live weights of samples of head lice, body lice and the F_1 and F_2 generations of hybrids are given in the two bottom columns of Table 6. These figures support the evidence of the linear measurements; the F_1 generation is intermediate in size, while the F_2 generation is indistinguishable from the B 24 strain in weight.

(4) *Biological observations on 'captive' strains*

The experiments to be described were all made during the summer of 1943 with head lice taken from the H 24 strain (being the 5th–8th generations in captivity) and body lice taken from the B 12 strain during the same period. The observations can conveniently be divided into bionomic data and miscellaneous experiments, the latter including resistance to starvation, mating choice and egg-laying preferences.

(*a*) *Bionomic data*

(i) *Egg stage.* Six batches of eggs from the body louse strain and six lots from the head louse strain were incubated at 30° C. and 75 % relative humidity. The percentages hatching on the subsequent days are shown in Table 8.

It will be noted that the incubation period of the two forms is very similar, but that the body louse eggs reach a higher final percentage hatch.

(ii) *Nymphal stages.* The following experiments were undertaken to compare the speed of development of the two strains in captivity. Larvae from louse eggs hatched in the incubator could be of different ages up to 24 hr. old and it was thought that this might affect the mortality during the first feed through gauze. (Partly starved lice, especially of the head strain, are unable to feed in this way.) Accordingly the experiments were all started with such lice as were able to take a meal in the breeding tin but were not more than 1 day old. From twenty-five to one hundred lice were used for each test and several tests were repeated twice.

The breeding tins were worn for different periods of the 24 hr. in different experiments, always keeping the appropriate head and body pair together. When not being worn, the tins were kept at room temperature. The results can best be shown diagrammatic-

ally and are set out in Figs. 6 and 7 which show the percentages moulting on different days and the pre-imaginal mortality of the two strains under different feeding conditions. Some of the salient points of the results are also recorded in Table 9.

The conclusions to be drawn from these results are rather similar to those arising from the egg data. The speed of development of the two forms is very similar; that is to say the various moults occur at similar intervals which vary according to the daily feeding period. The pre-imaginal mortality, however, is markedly higher in the head strain.

(iii) *Adult stage.* Adult lice were maintained under the same conditions of feeding as those described for the nymphal stages. Not more than two dozen adults were kept in a single tin and the females were kept slightly in excess of the number of males, since Buxton (1940) has shown that when females are in the presence of numerous males their length of life is shortened and the number of eggs per day decreases. Tapes carrying eggs were removed daily and incubated. By these means, it was possible to determine the individual lengths of life, the average numbers of eggs laid per female and the percentage hatch of the eggs under different conditions.

The overall means calculated from these data are given in Table 10. In this table it can be seen that, as Bacot showed, body lice live longer and lay more eggs per day than head lice. As regards the effect of different feeding periods per day, the length of life responds differently to egg production. Presumably owing to warmer average conditions, the metabolism of lice worn continually on the body is more rapid than lice fed 12 hr. per day; consequently the life span is exhausted earlier. Lice fed only 3 hr. per

Table 6. *Mean weights (in mg. with standard errors) of lice from 'captive' strains*

Date of sample	Strain	Generation	Males	Females
Nov. 1943	B24	6th	1·49 ± 0·051	2·40 ± 0·063
	H24	14th	0·90 ± 0·053	1·80 ± 0·017
Feb. 1944	B24	9th	1·39 ± 0·036	2·36 ± 0·101
	H24	18th	0·88 ± 0·030	1·64 ± 0·119
Aug. 1944	B24	19th	1·38 ± 0·040	2·41 ± 0·136
	H24	28th	0·84 ± 0·018	1·33 ± 0·033
Nov. 1944	B24	23rd	1·38 ± 0·059	2·36 ± 0·066
	H24	32nd	0·88 ± 0·025	1·29 ± 0·064
	(B × H) 24	1st	1·12 ± 0·020	1·73 ± 0·089
Dec. 1944	B24	24th	1·43 ± 0·033	2·49 ± 0·033
	H24	33rd	0·96 ± 0·018	1·36 ± 0·031
	(B × H) 24	2nd	1·47 ± 0·032	2·49 ± 0·156

Table 7. *Mean dimensions (in mm. with standard errors) of body strain, head strain and the 1st and 3rd generations of a cross between them. All fed 24 hr. per day*

Strain	Head length	Head width	Total length
	Males		
Body	0·5303 ± 0·0140	0·3907 ± 0·0020	3·380 ± 0·070
B × H Cross F_1	0·4909 ± 0·0073	0·3867 ± 0·0025	3·127 ± 0·030
B × H Cross F_3	0·5250 ± 0·0093	0·3905 ± 0·0032	3·350 ± 0·038
Head	0·4573 ± 0·0029	0·3618 ± 0·0022	2·786 ± 0·054
	Females		
Body	0·5555 ± 0·0052	0·4203 ± 0·0022	3·811 ± 0·036
B × H Cross F_1	0·5347 ± 0·0089	0·4209 ± 0·0035	3·708 ± 0·034
B × H Cross F_3	0·5575 ± 0·0063	0·4263 ± 0·0032	3·867 ± 0·038
Head	0·4737 ± 0·0030	0·3875 ± 0·0020	3·270 ± 0·022

Table 8. *Percentage hatch of batches of head lice and body lice incubated at* 30° C. *and* 75% R.H.

	Day of incubation					
Strain	7th	8th	9th	10th	11th	12th
Body lice	0	45	95	97	97	97
	0	10	81	99	99	99
	0	24	79	99	99	99
	0	12	63	97	97	97
	0	1	58	94	95	95
	0	31	91	96	96	96
Average	0	21	78	97	97	97
Head lice	0	50	83	85	85	85
	0	9	57	73	73	73
	0	19	64	80	83	83
	0	14	56	77	85	87
	0	5	60	87	91	91
	0	31	76	86	86	86
Average	0	24	66	81	84	84

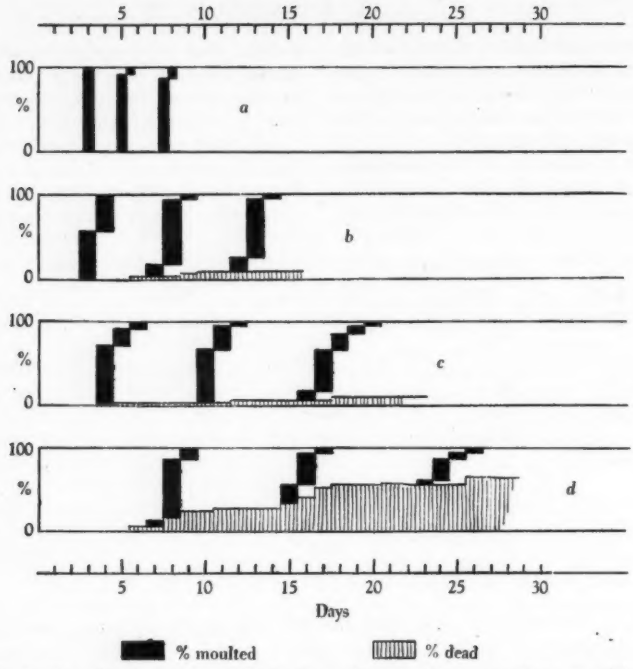

Fig. 6. Diagram illustrating development of body lice, showing the proportions moulting and dead. *a*=fed 24 hr./day; *b*=fed 12 hr./day; *c*=fed two periods of 3 hr./day; and *d*=fed 3 hr./day.

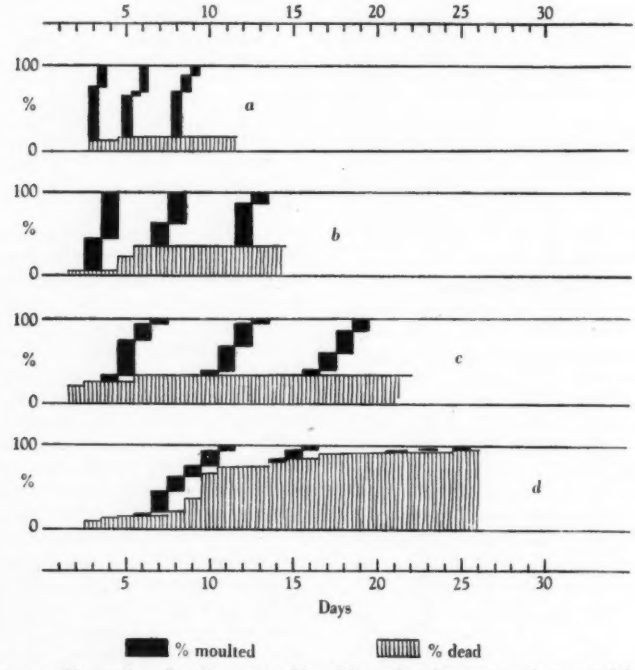

Fig. 7. Diagram illustrating development of head lice, showing proportions moulting and dead (*a*, *b*, *c* and *d* as in Fig. 6).

day, however, suffer from reduced nutrition which also curtails the life span. The optimum seems to be between 12 and 6 hr. feeding per day.

Daily egg production is directly dependent on the length of the daily feeding period; and this effect is so marked that the 24 hr. fed lice lay a greater total number of eggs per female despite the shorter average life.

(iv) *Additional experiments with a fresh body louse strain.* When these measurements of nymphal and adult life had been completed, it was perceived that the comparisons between the head strain and the body strain suffered from the anomaly that the latter had been maintained in culture for much longer than the former. Accordingly, a fresh culture of body lice was obtained from an infested man and, after four generations in captivity, experiments were done to determine the bionomic factors of the new strain. The results were as follows: (*a*) A batch of seventy-five 1st-stage larvae fed for 6 hr. per day moulted after averages of 5·0, 11·3 and 19·0 days. The total pre-imaginal mortality was 8 %. (*b*) A batch of twenty-five adults (twenty-one females and four males) were fed throughout life for 12 hr. per day. The average adult life of the females was 23·6 days; they laid 5·7 eggs per female per day over the optimum period and 108 eggs per female altogether of which 82 % hatched.

It will be found that in both these experiments, the development, adult life and egg production of the new strain was extremely similar to the ordinary body louse stock (B 12).

(*b*) *Miscellaneous experiments*

(i) *Resistance to starvation.* Adult lice of various strains were starved in incubators at 23 and 30° C. at 75 % relative humidity and in darkness. The insects were placed on pieces of tape in small vials and each was only examined for mortality once at the stated time. The percentages dead after the different intervals are shown in Table 11. These figures were calculated from rather small numbers (about ten lice of each sex), but the differences between the head and body forms are obviously very great. Rough curves drawn through these points give the following approximate periods for 50 % starvation:

	23° C.		30° C.	
Strain	Males (hr.)	Females (hr.)	Males (hr.)	Females (hr.)
Head lice	55	40	34	24
Body lice	86	80	45	36

Table 9. *Average times from hatching to various moults of head and body strains of lice worn for different periods of the day*

Strain	Worn on body (hr. per day)	Average time (days) from hatching to 1st moult	2nd moult	3rd moult	Pre-imaginal mortality %
Body lice	24	3·1	5·3	8·3	0
	12	3·4	7·9	12·8	9
	2×3*	4·4	10·3	17·5	9
	3	8·0	15·8	24·3	64
Head lice	24	3·3	5·6	8·5	15
	12	3·5	7·6	12·2	35
	2×3*	5·3	11·5	17·6	32
	3	8·3	15·4	23·0	97

* I.e. 3 hr. in morning and 3 hr. in evening.

Table 10. *Statistics of adult life of head and body strains of lice. The figures in parentheses in the 'length of life' columns give the numbers of individuals from which the averages were calculated. Standard errors are also given in these columns*

Strain	Fed (hr. per day)	Average length of life (days ± S.E.) Males	Females	Eggs* per female/day	Total eggs/female	Average percentage hatch of eggs
Body lice	24	19·6 ± 1·41 (16)	19·8 ± 2·74 (9)	11·1	110	94
	12	30·4 ± 2·43 (14)	20·6 ± 3·04 (11)	5·7	98	91
	2×3	30·1 ± 1·38 (22)	24·8 ± 1·31 (46)	3·4	73	78
	3	13·2 ± 2·60 (10)	15·4 ± 2·81 (9)	1·7	15	0
Head lice	24	10·5 ± 0·65 (4)	9·1 ± 0·81 (9)	7·5	57	88
	12	—	22·1 ± 3·17 (9)	4·3	56	76
	2×3	—	17·3 ± 2·19 (9)	2·6	22	64

* Over main oviposition period.

(ii) *Mating choice.* In order to determine whether the head and body strains exhibit any preference for mating with their own strain, experiments were conducted in which the two forms were confined together in the same breeding tin and examined for mating at intervals. In order to determine the origins of pairs in copulation, some simple marking method was necessary. In spite of several attempts, no paint or lacquer was found which would persistently adhere to the cuticle of the louse. Therefore they were marked by amputation of one antenna. (Sometimes the left antennae of body lice and the right of head lice, and sometimes vice versa.) After a day to recover from the operation, the experiment was started with equal numbers of both forms. The matings observed in the course of seven experiments are recorded in Table 12. This table is of considerable interest. The totals of like and unlike matings are not very different (twenty-two and twenty-five respectively); but among the unlike matings there is a great preponderance of matings between body males and head females. A possible explanation of this fact (and the corresponding low number of reverse matings) is the position at copulation in which the male has to arch his abdomen upwards to effect the union. Such a position is probably easier for males with a *relatively* larger abdomen, which might explain the larger number of successful matings between the larger (*corporis*) males with smaller (*capitis*) females.

(iii) *Egg-laying preferences.* The following simple experiments were conducted to compare the egg-laying instincts of the two forms of lice. Large breeding tins were furnished with tufts of human hair, pieces of black voile and pieces of black tape. About a dozen adult lice (either of *capitis* or *corporis* strain) were put in each tin and the tins were worn on the leg for 24 or 48 hr. The eggs laid on the various materials were then counted, with the results shown in Table 13. It will be seen that there was considerable variation from one tin to another, but that the head lice showed a clearly marked aversion to laying eggs on the fibres of tape when other materials were available. (If they have no other choice, they will lay eggs on tape provided in an otherwise bare tin.) With both strains, the averages suggest a slight preference for human hair over voile, but this is of doubtful significance in view of the small number of tests.

Table 11. *Results of starvation tests with body lice and head lice. The body lice used were from* B 24 *(8th generation) and stock (duplicate tests pooled) and the head lice were* H 24 *(17th generation)*

Temperature	Sex	Strain	Percentage dead after different periods (hr.) 24	41	48	72	96	120
30° C.	Males	Body lice	0	0	70	—	—	—
		Head lice	0	100	100	—	—	—
	Females	Body lice	0	90	100	—	—	—
		Head lice	100	100	100	—	—	—
23° C.	Males	Body lice	—	—	0	29	77	71
		Head lice	—	—	43	91	100	100
	Females	Body lice	—	—	0	12	95	100
		Head lice	—	—	71	100	100	100

Table 12. *Matings observed between head and body lice marked by amputation of one antenna*

Test no.	No. of pairs	Matings observed H♂ H♀	H♂ B♀	B♂ H♀	B♂ B♀
1	8	1	0	4	3
2	2	1	0	0	1
3	16	4	1	3	3
4	?	0	1	2	1
5	10	1	1	7	2
6	3	0	0	1	1
7	10	1	0	5	3
Total	—	8	3	22	14

(5) *Discussion of experimental section*

A variety of measurements of 'wild' head and body strains of lice have been given, including measurements of parts of the body, of the opercula of the eggs and of body weights. These measurements show that it is possible to find a variety of characters in which populations of the two forms of lice differ; but in all cases there are overlaps in the curves of distribution among individuals. From the systematic point of view, therefore, it is not possible to assign an individual to either race. However, Nuttall's statement (1919*b*) that 'typical *capitis* and *corporis* represent the extremes in the variation of one species *Pediculus humanus*. . .' is somewhat misleading, inasmuch as the two types are massed round quite distinct means.

The results of rearing head lice in captivity for 2 years proves that it is possible to breed this strain for about forty generations without any signs of

acquiring *corporis* characters. It must be admitted that the conditions (worn 24 hr. per day) were more favourable than those experienced by Bacot's strain (worn at night only). It seems not impossible that a *corporis* type could have been bred from Bacot's stock by natural selection (in the 2 or 3 years mentioned) since the *capitis* form is clearly a less efficient type. That such a change should have happened in 'four or more generations' seems unlikely, except with a strain which had been recently crossed with *corporis*. The measurements made on hybrids suggest either that *capitis* forms are rapidly weeded out, or that the *corporis* characters are dominant.

It has long been known that head louse-body louse hybrids are fertile for at least three generations and I have found no trace of a preference for homogeneous matings. The question then arises of the frequency of cross-mating in nature. Nuttall, Hase and Sikora all speak of this possibility and record many cases of people infested with both head and body lice. Such opportunities for cross-mating would inhibit the development of separate varieties, which can only evolve in comparative genetic isolation.

It seems possible to me that, in recent years, the two types of infestation are becoming separated by affecting different types of host. The head louse has been shown to be most prevalent among young people, especially girls. A very high proportion of these are free from body lice, which seem to be largely restricted to those living a vagrant life among casual wards and common lodging houses. Accordingly the body louse hosts are frequently men and usually older; the very bad cases are often elderly, somewhat incapable men, living a solitary life.

Table 13. *Results of tests showing egg-laying preferences of head and body strains*

Strain	Test	Eggs laid on different materials: Human hair	Voile	Tape
Body lice (B24)	I	70 (52%)	44 (32%)	22 (16%)
	II	66 (93%)	8 (7%)	0 (0%)
	III	36 (29%)	70 (56%)	18 (15%)
	IV	51 (40%)	18 (15%)	57 (45%)
	Average	— 53%	— 28%	— 19%
Head lice (H24)	I	62 (43%)	81 (57%)	0 (0%)
	II	145 (78%)	38 (22%)	0 (0%)
	III	60 (39%)	93 (61%)	0 (0%)
	IV	143 (80%)	36 (20%)	0 (0%)
	Average	— 60%	— 40%	— 0%

Table 14. *Measurements of temperature of the environment of head and body lice. 'Head temperature' taken among hair on the crown of the head. 'Body temperature' taken half way down side of the thorax. Both at 3 mm. from skin*

Subject	Indoors, air temperature 18·5° C.: Head temp.	Body temp.	Outdoors, air temperature 13·0° C.: Head temp.	Body temp.	Outdoor clothes worn
Man A	31·1	33·6	31·2	30·5	Beret, no overcoat
B	30·1	31·5	29·6	32·5	Light raincoat, no hat
C	33·0	33·9	30·0	31·3	Raincoat and peaked cap
D	31·3	33·3	29·7	31·0	Felt hat, no overcoat
Woman E	31·1	33·8	30·0	32·0	No hat or coat
F	32·6	33·4	31·7	29·6	No hat, light overcoat
G	33·0	33·0	30·8	29·6	No hat, light overcoat

Whatever may be the ultimate fate of the two types of louse, whether they survive long enough to develop into distinct species, it is clear that at present they are not sufficiently different to claim that rank. Since they are fairly similar in biology (speed of development) and since the hybrids are fertile and vigorous, they are more similar than the two races of *Calandra oryzae* described by Richards (1944). Moreover, it does not seem possible to distinguish them on morphological grounds as well as *Cimex lectularius* and *C. columbarius* (Johnson, 1939).

However, on the evidence that I have presented, there seem grounds for believing that there are some genetic differences between the two forms and they

may perhaps be described as varieties. Since the typical form of head and body lice has remained recognizable since the days of Lineaus, there seem good practical grounds for retaining separate names, as follows:

Pediculus humanus humanus L.,
Pediculus humanus capitis De G.

SUMMARY

1. Measurements were made of the head length, head width, total body length, antenna length, length and breadth of 3rd antennal segment of head and body lice from natural infestations. In all measurements (except the last) the means were significantly different for the two forms, but in all cases there was considerable overlap.

2. Head lice reared continuously on the body for forty-three generations (over 2 years) did not change systematically in size. Body lice reared under similar conditions were likewise constant.

3. There was a highly significant difference between mean measurements of all the samples of head lice reared for up to 2 years on the body when compared with body lice reared for a similar period in the same way. The means for lice worn 24 hr. per day were less than those worn 12 hr. per day in both strains. An experiment showed that this is probably due to a difference in average temperature.

4. The mean live weights of head and body strains were even more distinct than linear dimensions on the several occasions when these were recorded.

5. Measurements of the dimensions of the opercula of the eggs of the two races and the numbers of air cells in them were distributed in the same way as the body measurements: that is, distinct means but overlapping individuals.

6. Ventral abdominal muscles in wild head lice are typically confined to segment 5. (The forty specimens examined adhered to this rule.) In wild body lice, muscles are usually also present in segment 4 as well.

7. Hybrids (female body × male head louse) were fertile for several generations. In size, the F_1 generation was intermediate, but the F_2 and F_3 generations became identical with the body louse strain.

8. Eggs of the body louse strain hatched at the same time as those of the head strain, but the body strain reached a significantly higher percentage hatch.

9. The times of moulting during development were studied in both races in relation to differing opportunities to feed (24, 12, 6 and 3 hr. per day). The two strains showed very similar reactions in regard to speed of development, but throughout there was a higher pre-imaginal mortality in the head strain.

10. Head lice were shown to be considerably more susceptible to starvation than body lice.

11. If adults of the two strains are mixed, there is no tendency towards homogeneous mating. Of the mixed matings, those between body louse males and head louse females are much more common than the reverse.

12. Female head lice show a slight difference in their egg-laying preferences when offered a choice of hair, voile and tape.

My thanks are due to Prof. P. A. Buxton, F.R.S., for a number of helpful suggestions based on his extensive knowledge of louse biology and to Dr J. O. Irwin for critical comments on the statistical issues.

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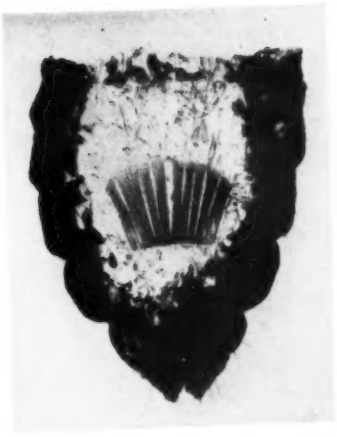
(*a*)



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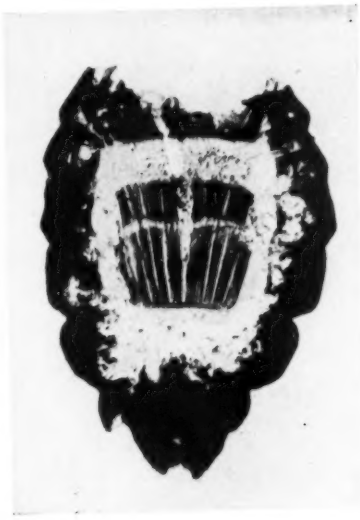

(*c*)

(*d*)

Dissections of lice to show ventral longitudinal abdominal muscles in the female. *a*, head louse; *b*, body louse with single set of muscles on segment 5; *c*, body louse with muscles on segments 4 and 5; *d*, abnormal body louse with muscles on segments 3, 4 and 5.

I.

1. Intro

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THE ECOLOGY OF IMMATURE PHASES OF TRICHOSTRONGYLE NEMATODES

I. THE VERTICAL DISTRIBUTION OF INFECTIVE LARVAE OF *TRICHOSTRONGYLUS RETORTAEFORMIS* IN RELATION TO THEIR HABITAT

By H. D. CROFTON, B.Sc., Ph.D.,* *King's College, Newcastle-upon-Tyne*

(With 5 Figures in the Text)

CONTENTS

	PAGE
1. Introduction	17
2. Vertical distribution of infective larvae	17
(i) Methods	17
(ii) Distribution on herbage	18
(*a*) *Festuca* spp.	18
(*b*) Clover	19
(*c*) *Carex vulgaris*	19
(iii) Distribution in soil, 'mat' and herbage	19
3. The habitat	19
(i) Conditions in grass	19
(*a*) Temperature	19
(*b*) Relative humidity	21
(*c*) Rate of evaporation	22
(*d*) Light intensity	22
(ii) Clover, *Carex* sp., *Poa* sp.	23
4. Discussion	23
5. Summary	24
References	25

1. INTRODUCTION

In the life cycles of Trichostrongyles the egg stage passes out in the faeces of the host and is deposited on the grass of the pasture. The first and second stage larvae normally remain in the faeces, and it is not until the third or infective stage is reached that migration from faeces to the grass blades occurs. The micro-climate of grass has an important part to play in the development, behaviour, distribution and survival of these free living stages. Obviously the third stage larvae will be affected most directly because they are in actual contact with the soil, the surface layers and the grass blades.

The importance of the habitat has been ignored by most workers; Mönnig (1930), Morgan (1938) and Taylor (1938) have made some general observations but no detailed study has been attempted. With the exception of Taylor (1938), workers have investigated the vertical distribution of Trichostrongyle larvae under laboratory conditions only. Ecology is the study of animals in relation to their environment, yet most workers in this particular field have studied the effect of arbitrarily-chosen factors on nematode larvae. Frequently the conditions in laboratory experiments have borne no relation to those found in the normal environment. The present work is an endeavour to consider the vertical distribution of infective larvae as an ecological problem and not as a study of animal behaviour.

2. VERTICAL DISTRIBUTION OF INFECTIVE LARVAE

Until recently it was believed that infective larvae migrated to the tips of grass blades and remained there in relatively large numbers until they either died or were eaten by a grazing animal (see Southwell & Kirshner, 1938). Taylor (1938) and later Kauzal (1941) showed that this was not so, and below is an account of a more detailed investigation into the vertical distribution as it occurs under natural conditions.

(i) *Methods*

Infective larvae of *Trichostrongylus retortaeformis* were used throughout these experiments. They were obtained from fresh faeces-charcoal cultures. These cultures were made by mixing equal quantities of faeces and animal charcoal and adding sufficient water to keep the mixture moist. The cultures were incubated at 24° C. The faeces was obtained from laboratory rabbits in which a pure infestation of *T. retortaeformis* had been established. Larvae were separated from the cultures on clean moist filter paper on to which they migrated from the faeces-charcoal mixture.

When large numbers of larvae were counted, a dilution sampling method was used, samples being taken with a Stoll pipette (Stoll & Hausheer, 1926). The volume of water in which the larvae were

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suspended was reduced to 10 c.c. after centrifuging. The 10 c.c. of suspension were then thoroughly mixed and ten samples of 0·15 c.c. each were taken. The number of larvae in each of these 0·15 c.c. samples was counted. Occasionally a series of results was examined statistically to ensure that the technique employed was giving random samples. Smaller numbers (less than 500) of larvae were counted individually.

Temperatures were taken with thermometers checked against standard instruments; a hair hygrometer was used to measure humidities, a wet and dry bulb thermometer being used for calibration; the rate of evaporation was estimated with Piché evaporimeters, the two used being compared before the experiments commenced. Light intensities were recorded photo-electrically by a Weston Light Meter; where possible, single readings were not made, most figures quoted being the mean of four readings taken in the same plane facing north, south, east and west.

Table 1. *The number of larvae on* Festuca *spp.* (5·5 *in. high*) *at different levels on three successive days*

		24 hr.			48 hr.			72 hr.		
Level	Plot ...	1	2	3	4	5	6	7	8	9
(A) Top inch		4	6	1	8	2	3	4	7	12
(B) 1·5 in. below (A)		54	63	51	61	74	84	67	52	49
(C) 1·5 in. below (B)		301	330	342	391	363	362	418	388	421
1·5 in. below (C)		369	383	361	405	397	368	402	453	438
Total		728	782	755	865	836	817	891	900	920

Table 2. *The number of larvae* on* Festuca *spp.* (3 *in. high*) *at different levels on three successive days*

	24 hr.			48 hr.			72 hr.		
Level Plot ...	1	2	3	4	5	6	7	8	9
Top inch	1	0	0	2	0	1	0	0	0
Middle inch	24	30	23	39	28	31	27	33	29
Lower inch	330	295	307	347	381	362	377	381	365
Total	355	325	330	388	409	394	404	422	394

* No. of larvae initially placed on each plot = 1000.

(ii) *Distribution on herbage*

The experiments described below were repeated several times during the summer of 1942, but only details of single experiments are given. The details quoted are representative of the series of experiments and no fundamental difference in distribution was found in any of the other experiments, although the total number of larvae migrating up the blades was different at different periods.

(*a*) *Festuca* spp. Nine plots of *Festuca* spp., 4 in. square, were marked with metal strips in an open garden. The plots were not shaded or protected by trees or buildings. The grass, which had been allowed to grow, was then trimmed to a uniform height of 5·5 in. Nine sets of 2000 infective larvae of *T. retortaeformis*, each in 10 c.c. water, were prepared. One set was used for each plot, and the suspension of larvae scattered as evenly as possible over the soil at the base of the grass blades by means of a fine pipette.

Twenty-four hours later the grass on three plots was cut with scissors, the top inch being removed first and the remaining part of the blades cut so that three lengths of approximately 1·5 in. each were obtained. This gave twelve samples of grass from three plots. The larvae were washed off each sample separately and the numbers counted. Three of the remaining plots were examined similarly after 48 hr., and the last three after 72 hr. The results are given in Table 1.

To determine whether the distribution of larvae was limited by the distance they were able to move, the above experiment was repeated using plots of *Festuca* spp. trimmed to a height of 3 in. Results are given in Table 2.

Tables 1 and 2 show that with grass blades 5·5 in. in length approximately 50 % of the total number of larvae on the blades occurred above the basal 1·5 in.; with blades of only 3 in. in length approximately 90 % of the larvae were found on the inch of grass nearest the soil. Conditions were very similar during these two experiments, so that it is clear that the height to which the larvae climbed was not limited by the total distance they were able to travel. Nor was their distribution governed by their rate of movement only, since, although there was a slight increase in the number of larvae on the blades after three days, the percentage distribution per inch was little altered.

(*b*) *Clover*. Experiments similar to those described above were made with clover. Only two plots per day were used and 1000 larvae distributed over each plot. Examinations were made after 24, 48 and 72 hr., the leaf and the upper and lower halves of the stem being examined separately. Results are shown in Table 3.

Table 3. *The number of larvae on clover at different levels on three successive days*

	24 hr.		48 hr.		72 hr.	
Level Plot ...	1	2	3	4	5	6
Leaf	158	173	301	312	344	320
Upper ½ stem	291	276	263	245	330	353
Lower ½ stem	307	319	333	313	321	339
Total	756	768	897	870	995	1012

After 24 hr. 75 % of the larvae placed on the plot were on the plant, but most of these were on the stems, only 20–25 % of them being found on the leaves. After 48 hr., however, the larvae were evenly distributed over the stems and the leaves.

(*c*) *Carex vulgaris*. Experiments similar to those described above were carried out with plots of *C. vulgaris* 10 in. in height. Results are given in Table 4.

Table 4. *The number of larvae on* Carex vulgaris *(10 in. high) at different levels on three successive days*

	24 hr.		48 hr.		72 hr.	
Level Plot ...	1	2	3	4	5	6
A=Upper 4 in.	0	0	0	0	0	0
B=2 in. below A	0	0	0	0	0	0
C=2 in. below B	0	0	0	1	0	0
D=lower 2 in.	110	89	73	102	65	86

Here it will be seen that larvae were limited to the lower 2 in. of the plant.

(iii) *Distribution in soil, 'mat' and herbage*

On a pasture there are three layers: the soil, the herbage and between these a layer which can conveniently be called the 'mat'. The 'mat', which varies in thickness from field to field, mainly consists of dead plant material lying at the soil surface; its thickness depends upon the type of vegetation and the age of the pasture. It is important in the control of moisture loss from the soil surface, and must necessarily have some effect on the temperature of the underlying soil. The herbage encloses a layer of air which is relatively still and partially insulated from the air above. Obviously the longer and more densely growing the herbage is, the greater will be the differences between the microclimate and the air above.

A plot of ground uniformly covered with grass, mainly *Festuca* sp., was marked out with metal strips to make plots 4 in. square. The grass on each plot was trimmed to a uniform height of 3 in. immediately before it was used. Each month, during the period June 1941–May 1942, 15,000 third-stage larvae of *Trichostrongylus retortaeformis* were pipetted on to the soil at the base of the grass blades on one plot. Seven days after the inoculation of a plot the grass was cut, leaving the lower quarter of an inch, which was surrounded by the debris forming the 'mat'. The 'mat' and the remaining quarter of an inch of the grass blades were then removed. The first inch of soil, now exposed, was then collected. From these three samples the larvae were removed by Baermann's technique, washing and centrifuging; the number from each sample was counted separately.

Fig. 1 shows the number of larvae recovered each month from the grass blades, the 'mat' and the soil. The number of larvae in each case is expressed as a percentage of the total number recovered.

During the warm months of June, July and August the majority of larvae found were on the grass blades and relatively few were found in the 'mat'. In this period there were more larvae found in the soil than in the 'mat'. During September, October, April and May fewer larvae were found in the soil and on the grass blades and more in the 'mat'. In the period November to March inclusive almost all the larvae were recovered from the 'mat'; in the coldest period, December to February, no larvae were recovered from the soil or the grass blades.

Thus, during the cold period the larvae remained at the site of inoculation and no upward migration occurred because of inactivity of the larvae and not because of downward migration into the soil. This is not in agreement with the findings of Kauzal (1941), who found that the majority of larvae remained in the soil in cool weather (45–60° F.). He, however, divided the habitat into only two sections—grass and soil, his figures for soil are probably equivalent to the present figures for soil and 'mat' combined.

3. THE HABITAT

Some of the conditions occurring in the 'mat' and herbage were studied and the results are given below. It must be emphasized that the factors governing the microclimate are complex and interacting, so that no precise or complete summary of them can be given, but the general trends of some of them may be followed.

(i) *Conditions in grass*

(*a*) *Temperature*. Measurements of temperature were made at different levels in grass on various days in the two periods 1–21 August 1941 and 3–15 October 1941. Figs. 2 and 3 summarize the results.

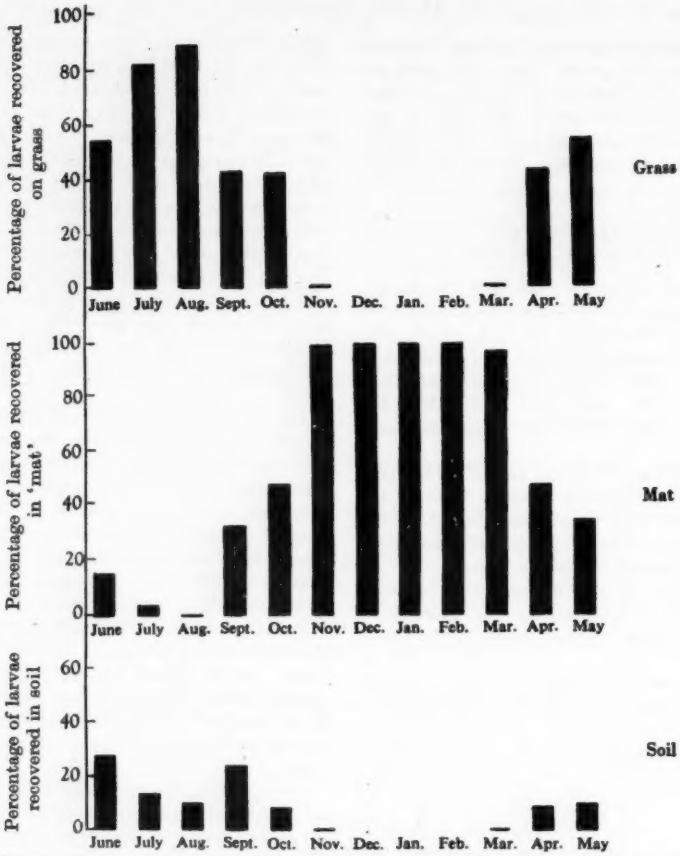

Fig. 1. Showing relative numbers of larvae recovered from grass blades, 'mat' and soil throughout the year June 1941–May 1942.

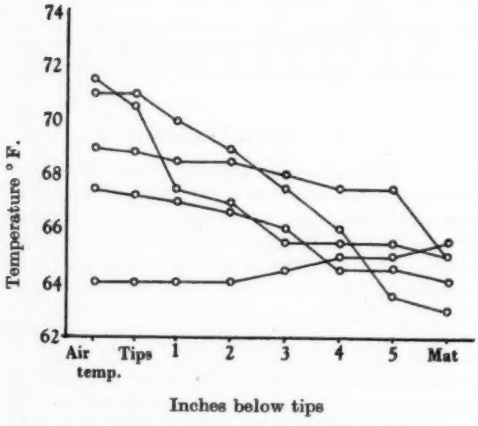

Fig. 2. Showing temperature at different levels in *Poa* grass 6 in. high, Aug. 1–21, 1941.

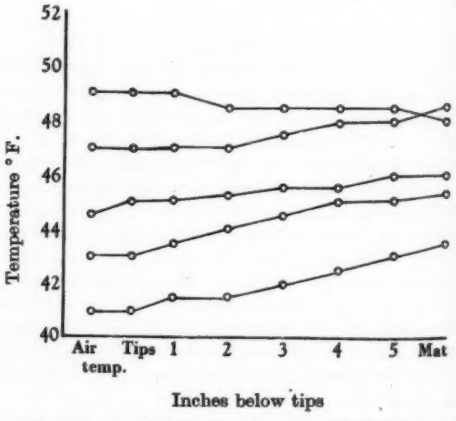

Fig. 3. Showing temperature at different levels in *Poa* grass 6 in. high, Oct. 3–15, 1941.

From figures supplied by Dr A. Milne, Table 5 has been constructed to show the temperatures at the tips and bases of the blades of grass during the period 29 March–29 May 1941.

Figs. 2 and 3 and Table 5 show that the temperature below the top of the grass may differ considerably from that of the air above. Figs. 2 and 3 show that a gradient of temperature may be established between the upper and lower parts of the grass. pastures the amount of 'mat' is usually sufficient to maintain a high relative humidity at the base of the grass blades. Results of observations on the relative humidity at different levels in grass are shown in Fig. 4.

From figures supplied by Dr A. Milne, Table 6 has been constructed to show the relative humidity at tips and bases of grass blades on days during the period 29 March–29 May 1941. These readings

Table 5. *Temperatures at tips and base of blades of grass**

	Temp. in ° F.			Temp. in ° F.			Temp. in ° F.			Temp. in ° F.	
Date	Tips	Base	Date	Tips	Base	Date	Tips	Base	Date	Tips	Base
29 Mar.	39	40	11 Apr.	52	50	24 Apr.	44	45	10 May	59	60
30 „	52	49	12 „	54	55	25 „	61	54	13 „	68	67
1 Apr.	44	44	15 „	45	52	26 „	68	68	14 „	54	59
2 „	51	55	16 „	53	52	29 „	49	47	15 „	53	52
3 „	43	43	17 „	48	48	30 „	58	55	16 „	60	58
4 „	60	50	18 „	55	55	2 May	54	57	17 „	68	66
5 „	43	43	19 „	47	49	3 „	56	53	20 „	68	70
6 „	52	37	20 „	44	43	6 „	68	59	22 „	54	56
9 „	51	46	22 „	45	49	7 „	58	54	27 „	68	65
10 „	52	51	23 „	52	48	8 „	56	54	29 „	66	66

* Reading made between 2 and 4 p.m.

Table 6. *Relative humidities at tips and bases of grass blades**

	Rel. hum. (%)			Rel. hum. (%)			Rel. hum. (%)			Rel. hum. (%)	
Date	Tips	Base	Date	Tips	Base	Date	Tips	Base	Date	Tips	Base
29 Mar.	90	97	11 Apr.	72	85	24 Apr.	97	99	10 May	83	84
30 „	65	93	12 „	62	84	25 „	72	94	13 „	67	91
1 Apr.	75	86	15 „	55	97	26 „	52	84	14 „	84	85
2 „	49	65	16 „	48	65	29 „	89	96	15 „	90	98
3 „	89	97	17 „	82	86	30 „	95	96	16 „	55	62
4 „	51	91	18 „	47	94	2 May	77	82	17 „	75	93
5 „	77	94	19 „	80	97	3 „	76	84	20 „	51	85
6 „	60	80	20 „	87	89	6 „	55	91	22 „	90	95
9 „	57	83	22 „	89	99	7 „	65	94	27 „	80	92
10 „	54	69	23 „	84	94	8 „	91	99	29 „	85	93

* Readings made between 2 and 4 p.m.

From Table 5 it can be seen that the temperature difference between tips and base may be as great as 10° F. (4 April) or may be non-existent. The temperature at the tips may be higher (6 May) or lower (14 May) than at the base.

(*b*) *Relative humidity.* The almost closed system found between grass blades usually has a very different moisture content from that of the outside air. Observations were made at various times and it was noted that even during a prolonged drought of more than 3 weeks the relative humidity in the 'mat' was above 90 % on normal well-drained pasture. The retention of moisture must be governed by the height and density of herbage, the thickness of the 'mat' and the type of soil. Even in closely grazed were made with a hair hygrometer on a hill pasture. Readings of 95 % R.H. in Fig. 4 represent R.H. 95–100 %, the hygrometer being inaccurate over this range.

Fig. 4 and Table 6 show that a high humidity occurred in the lower parts of the grass, even when the humidity of the atmosphere was low. Invariably the relative humidity at the base was higher than at the tips of the blades.

These observations are important in interpreting the temperature gradients existing between the soil and the tips of the grass blades. Since maximum humidity occurs near the soil surface or in the 'mat', the specific heat of the surface layers will increase towards the soil surface. This will result in a time lag

in the temperature changes in the lower parts of the herbage. With fluctuating air temperatures the grass temperature can rarely be the same as that of the air, but may be either higher or lower according to past conditions.

Table 7 shows that there was a decrease in the rate of evaporation towards the base of the grass blades. This might be expected from a study of the humidity conditions. It will be seen that the rate of evaporation decreased rapidly below

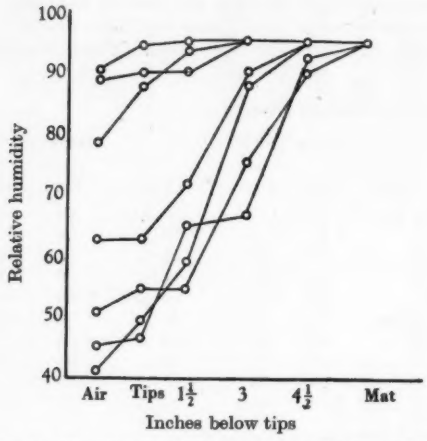

Fig. 4. Showing relative humidity at different levels in *Poa* grass on different days.

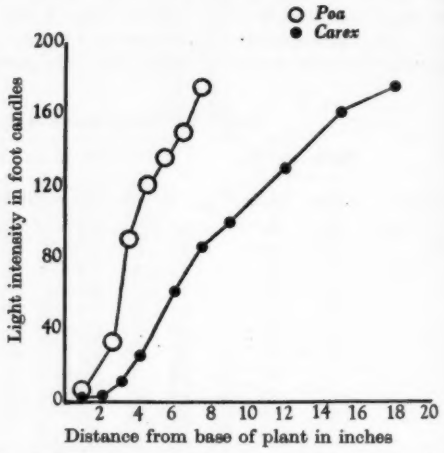

Fig. 5. Showing light intensities at different levels in *Poa* grass and *Carex*.

Table 7. *Comparison of the rate of evaporation at different levels in grass with the rate of evaporation in air for the same period*

Date 1941	Position of evaporimeter	Evaporation in c.c.	Evaporation in c.c. for same period	Ratio: Evap. in grass / Evap. in air
1–2 Sept.	Tips of blades	6·2	7·0	0·88
3–4 „	1–2 in. below tip	3·2	4·6	0·70
5–6 „	2–3 in. „ „	3·6	6·3	0·57
7–10 „	3–4 in. „ „	5·0	12·5	0·40
11–16 „	4–5 in. „ „	2·85	13·3	0·215
17–21 „	5–6 in. „ „ (Base)	0·1	15·5	0·006

(*c*) *Rate of evaporation.* This is governed by temperature, air humidity, wind and the type of surface at which evaporation occurs. In the lower levels of herbage the effect of wind can be neglected, since the intertwining blades almost completely shelter this region from wind disturbance. The rate of evaporation, therefore, is mainly dependent upon humidity and temperature.

Comparisons of the rates of evaporation at different levels on grass were made. Since only two Piché evaporimeters were available, the reading at any one level was compared with the rate of evaporation 2 ft. above the soil surface. Thus the readings given for different levels were taken at different times, but they may be compared by considering the rate of evaporation occurring at the same time at 2 ft. above the grass. Results are given in Table 7.

3 in. from the top of the blades. In the layer 5–6 in. below the tips (the 'mat') the rate of evaporation was negligible.

In general, therefore, there is a negative correlation between the humidity and the rate of evaporation at any level.

(*d*) *Light intensity.* The presence of grass blades must reduce the light intensity in the lower levels of the herbage. Several readings were taken and the results, comparing light intensity at different levels in *Poa* grass and *Carex*, are given in Fig. 5. These observations were made on a sunny day in September 1941. Other readings on different days show similar trends. There is a definite gradient from the top of the grass to the soil surface, and it will be seen that midway between the tips and the bases of the blades the light intensity falls rapidly.

(ii) *Clover*, Carex *sp. and* Poa *sp.*

The umbrella-like leaf of clover and the low straggling growth of the plant play an important part in governing the special microclimate formed by this type of herbage. The large, closely packed leaves form an almost continuous roof over the underlying stems so that these latter are surrounded by air which is almost completely insulated from the normal atmosphere.

In contrast with clover, forms like *Carex* have long stems and/or long lanceolate leaves which do not interlace. Here the narrowness of the leaf and the stem tend to make the difference between the climate above and that below the tops of these plants less marked.

Observations were made on *Carex vulgaris* and clover and *Poa* sp.

4. DISCUSSION

It has been stated that larvae migrate to the tips of blades of grass and remain there until eaten by a potential host (Southwell & Kirshner, 1938). This implies that the highest concentration of larvae should occur at the tips of the blades. The present work shows that larvae normally occur on the lower portions of the herbage, near the base of the blades and in the 'mat'.

Taylor (1938) showed that most of the larvae occurred in the lower parts of the grass, but he only modified former ideas to explain this distribution. He, and others, have retained the theory of migration to the tips of blades. Having shown that a high death rate occurs at the tips, Taylor explained the distribution on the basis of the death rate being low in the lower portions of the herbage, and high in the upper parts.

This still leaves the problem of explaining why the larvae 'exhibit negative geotropism'. Naming the movement 'negative geotropism' (or more correctly 'negative geotaxis') is no solution, because the terms cannot be accepted literally in relation to these larvae. They cannot be accepted because in thin films such as are found on grass blades, the force of gravity acting on the very small larvae is negligible in comparison with the surface tension effects. Payne (1923*b*) discussed the factors influencing the migration of larvae of *Necator americanus* in soil, and showed that the factors which affected surface tension of water surrounding the soil particles affected the migration of the larvae. She recognized that the forces of gravity on the larvae could be neglected where the spaces between the soil particles were not filled with water, that is, when the larvae were in a film of water. She was careful not to describe the movement of the larvae as 'geotaxis' and investigated the factors influencing the migration. The two habitats, soil and the surface of grass blades, differ considerably in their physical properties, but there is a general similarity in many respects, mainly in the presence of surface films of moisture.

Let us now consider the factors which influence the migration of larvae up the grass blades.

The present experiments, as well as those of Kauzal (1941), have shown that migration downwards always accompanies migration upwards; the only time no downward migration occurs is when the larvae do not move from the 'mat'.

We may postulate, then, that there is a random movement of larvae. The reason why little lateral migration occurs is probably because there is little fluctuation of conditions in a horizontal plane; but vertically there are, as has been shown, very strong gradients of temperature, humidity, rate of evaporation and light intensity. Thus, stimulation will occur in one plane, that is, vertically. The higher proportion of larvae on the blades as compared with the number in the soil can be explained reasonably on the grounds that the impedance offered by the surface of grass blades is smaller than that offered by the soil. Lucker (1938), working on horse strongyles, and Payne (1922, 1923*a*, *b*) working on *Necator americanus* have shown that the rate of migration through soil varies according to the type of soil and that heavy clay soils can prevent migration entirely.

One must look, however, for other factors to explain the distribution on the blades themselves. It has been shown previously that the height of the grass blades, and the type of herbage, alter the extent of scatter of larvae along the length of the blade, so that this cannot be explained on the basis of random movement. It must be noted equally that it cannot be explained by considering the movement as geotropism. Veglia (1915), working on *Haemonchus contortus*, Mönnig (1930), working on Trichostrongyles, and others, have shown that movement of larvae only occurs in the presence of moisture. In a previous section it has been shown that high humidities occur in the lower half of the grass, whatever the humidity of the atmosphere may be. Also the larvae are almost entirely restricted to this lower portion. Where the humidity is high near the tip, as it is in clover, and possibly in grass provided that the air humidity is high, the larvae occur on the upper portions of the herbage. Temperature affects the activity of larvae (Mönnig, 1930; Rogers, 1940; Buckley, 1940) and higher temperatures might be expected to increase the rate of migration, but the extent of the migration must always be limited by the amount of moisture. Because, however, changes in humidity and temperature are intimately related, the effect of temperature must be a complex one. Considerable differences in temperature may occur between the tips and bases of the blades, but the

lower portions of the herbage are not subjected to extreme or rapid changes of temperature.

There have been several observations made on the influence of light intensity on the activity and migration of larvae (see Veglia (1915), Cameron (1923), Mönnig (1930), Rogers (1940), Buckley (1940)). Most workers agree that a diffuse light of low intensity is favourable to migration. Rogers (1940) showed that movement occurred only at light intensities of 15–155 f.c., maximum activity occurring at 62 f.c. From the observations described above, a light intensity of 60 f.c. occurred near the middle of the plant on sunny days in September. On brighter days this intensity would be found nearer the base. The light intensity which is most favourable for movement, therefore, will be found in the lower portions of the herbage and this is normally where the larvae are found in the greatest numbers.

Changes of temperature, humidity, and the rate of evaporation will, however, not affect the larvae alone. Payne (1923*b*) has discussed the effect of these factors on water movements and on the change in thickness of surface films. The changes in the surface film also influence the direction and rate of movement of the larvae.

Briefly then, the distribution on herbage may be explained by postulating a random movement of the larvae, this movement being restricted downwards by the impedance offered by the soil, and upwards by climatic factors. The extent of the spread upwards is limited by the moisture film on the grass blades and this is directly correlated with the gradient of humidity which occurs between the soil surface and the top of the grass. The thickness of the moisture film is altered by changes in the other climatic factors (Payne, 1923*b*). Temperature, which influences the activity of these larvae, varies least nearest the base of the herbage. The larvae, therefore, are found in greatest number where most constant temperature conditions occur. In the lower portions of the herbage the intensity of light and the amount of diffusion is most favourable for movement of the larvae.

The larvae, therefore, are distributed in that portion of the herbage where there is least climatic change, the type, height and density of the herbage modifying the effect of climatic factors which influence the extent of their movement.

5. SUMMARY

1. The number of third stage larvae of *Trichostrongylus retortaeformis* on different portions of herbage was studied. Three plant-types were used: *Festuca* sp., Clover, and *Carex* sp.

2. On *Festuca* spp., 5·5 in. in height, 90 % of the total number of larvae occurred on the lower 3 in. of the blades; 50 % occurred on the basal 1·5 in. On the same species, 3 in. high, 90 % were found on the lower inch. This showed that the distribution on the blades was not limited by the total distance the larvae were able to travel. Nor was it limited by the rate of movement, because the form of the distribution was not altered after 3 days.

3. Larvae were found to be evenly distributed vertically over the stems and leaves of clover.

4. On *Carex vulgaris* 10 in. high the larvae were limited to the lower 2 in.

5. The distribution of larvae in the soil, 'mat' and herbage was recorded monthly throughout the year. During the warmer months of June, July and August the majority of larvae were found on the grass blades, and more larvae occurred in the soil than in the 'mat'. During September, October, April and May more larvae were found in the 'mat' and fewer were found on the blades and in the soil. From November to March nearly all the larvae occurred in the 'mat'. From December to January no larvae were found on the grass blades or in the soil.

6. Climatic conditions in the herbage were studied.

7. It was shown that the temperature below the top of grass blades may differ considerably from the air temperature. Differences of 10° F. were recorded between the tips and bases of grass blades. In general, there was a gradient of temperature established between the upper and lower parts of the grass.

8. High humidities occurred in the lower parts of the grass, even when atmospheric humidity was low. The relative humidity at the tips was never higher than that at the base of the blades.

9. The rate of evaporation decreased towards the base of the blades.

10. The light intensity decreased towards the soil. Midway between the top of the grass and the soil the light intensity fell rapidly.

11. The height, density and type of herbage modified the effect of the climatic factors.

12. It was concluded that the vertical distribution of larvae could be explained without reference to geotropism and that most of the larvae occurred in that portion of the herbage in which there was least climatic change.

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THE ECOLOGY OF IMMATURE PHASES OF TRICHOSTRONGYLE NEMATODES

II. THE EFFECT OF CLIMATIC FACTORS ON THE AVAILABILITY OF THE INFECTIVE LARVAE OF *TRICHOSTRONGYLUS RETORTAEFORMIS* TO THE HOST

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(With 6 Figures in the Text)

CONTENTS

	PAGE
1. Introduction	26
2. Methods	26
3. Hatching of eggs and development of larvae	27
(i) Rate of hatching and development	27
(ii) Survival	27
(*a*) Dry conditions	27
(*b*) Winter conditions	27
4. Activity and survival of infective larvae	29
(i) Daily fluctuations in the number of larvae on grass	29
(ii) The rate of disappearance of larvae	32
5. Discussion	36
6. Summary	37
References	38

1. INTRODUCTION

It is well known that the number of eggs produced by trichostrongyles and other helminths is much greater than the number of adults which develop from them. Not only do many eggs fail to produce first-stage larvae, but many of the non-parasitic larvae either die or fail to enter the host. In a previous paper (Crofton, 1947) observations have been recorded on the environmental factors which influence the behaviour of the infective larvae. This paper records observations made upon the climatic factors which influence the hatching of the eggs and upon the activity and survival of the larvae.

The term 'availability' refers in this paper only to the position and numbers of larvae on grass blades. It has been assumed that the host normally eats only the grass blades above the 'mat' (see Crofton, 1948). This is probably true for most grass-eating animals but the work presented here is only concerned with infestation of the rabbit with *Trichostrongylus retortaeformis*. Grazing habits of sheep in relation to infestation will be discussed in another paper.

2. METHODS

Ova and larvae of *T. retortaeformis* were used throughout these experiments. The ova were obtained from laboratory rabbits in which a pure infestation of *T. retortaeformis* had been established.

In experiments on the hatching and survival of eggs, the faecal pellets were collected and used within 3 hr. of being passed by the rabbits. The number of eggs used in experiments was estimated by taking a sample of each collection and counting the numbers of eggs per g. of faeces, using the McMaster cell method (Gordon & Whitlock, 1939). Larvae were obtained from faeces-charcoal cultures as described in a previous paper (Crofton, 1948); they were separated from the culture by allowing them to migrate on to clean moist filter paper. When large numbers of larvae were counted, a dilution sampling method was used, samples being taken with a Stoll pipette (Stoll & Hausheer, 1926). The volume of water in which the larvae were suspended was reduced to 10 c.c. after centrifuging. The 10 c.c. of suspension were then thoroughly mixed and ten samples of 0·15 c.c. each were taken. The number of larvae in each of these 0·15 c.c. samples was counted. Occasionally a series of results was examined statistically to ensure that the technique employed was giving random samples. Small numbers (less than 500) of larvae were counted individually. Larvae were removed from grass by washing and centrifuging.

Experiments were made on plots marked out on grass which had never been grazed by animals which could have harboured trichostrongyle parasites.

Temperatures were taken with thermometers checked against standard instruments; continuous recordings were made with a thermograph. A hair hygrometer and wet and dry bulb thermometers were used to measure humidity and a Piché evaporimeter used to record the rate of evaporation.

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3. HATCHING OF EGGS AND DEVELOPMENT OF LARVAE

It will be shown below that the problem under discussion is one of populations rather than of individuals, and each stage in the life history can only be studied in relation to the other stages. Thus the availability of the infective larvae to the host is primarily dependent upon the rate of hatching and survival of the ova.

(i) *Rate of hatching and development*

During the period June 1941–June 1942, a series of grass plot experiments were made to determine the effect of weather conditions on the hatching of eggs of *T. retortaeformis*. Each month during the experiment 30 g. of freshly passed faeces were collected from rabbits with a pure infestation of *T. retortaeformis*. The faeces were divided into six portions, A_1, A_2, B_1, B_2, C_1 and C_2 of 5 g. each.

Samples A_1 and A_2 were used as controls. The number of eggs per g. was determined from sample A_1, while sample A_2 was incubated at 24° C. and the number of larvae counted. Samples B_1, B_2, C_1 and C_2 were scattered over separate, but adjacent, plots of grass on which the blades had been trimmed to a height of 4·5 in. B_1 and B_2 were collected after 8 days' exposure and C_1 and C_2 after 15 days. B_1 and C_1 were used for egg counts. B_2 and C_2 were incubated and the number of larvae estimated.

From the results of each monthly experiment the percentage of eggs hatching after 8 days and after 15 days could be calculated. When B_1 and C_1 were examined the larvae present were noted and the degree of their development was identified. If all the eggs had hatched, and no larvae recovered, it was assumed that the larvae had reached the third stage and had migrated out of the faeces. No evidence was obtained to show that the first and second stage larvae normally leave the faecal pellet.

Recordings of temperature, humidity, rate of evaporation, sunshine and rainfall were made. Fig. 1 summarizes the results.

From June to October inclusive eggs hatched in 15 days or less. During July, August and September hatching occurred in 8 days or less. Under favourable conditions in the laboratory eggs hatch in 24–30 hr. after they have been passed, but, although eggs may have hatched in much less than 8 days during July, August and September, the experiment was not designed to determine actual times.

During June no eggs had hatched after 8 days (maximum temperature 50–55° F.) but 85 % of them had hatched after 15 days. From November to March, when maximum temperatures were below 50° F., no hatching occurred. When, in April, maximum temperatures rose to 50° F., no eggs hatched during 15 days, but when, in May, the temperatures were above 55° F. hatching occurred during 8 days.

Under the conditions of the experiment, therefore, maximum temperatures near 50° F. were critical for hatching. When maximum temperatures of 60–80° F. occurred, hatching took place in 8 days or less. There was no obvious correlation between the rate of hatching and the humidity, hours of sunshine, the rate of evaporation or the rainfall.

(ii) *Survival*

Two problems arose out of the observations made upon the hatching of the eggs. First, during periods when the rate of evaporation was high, hatching occurred and the larvae reached the infective stage. Examination of Fig. 1 shows this. Mönnig (1930), however, who worked in South Africa with species of *Trichostrongylus* parasitic in sheep, stated that drying of the faecal pellet on the pasture can kill the egg and the first and second larval stages. Secondly, from November to March, no hatching had occurred 15 days after the eggs had passed out of the host, but the eggs were not killed by short exposure to drastic influences during this period. Further work was necessary to determine whether eggs laid on pasture remained viable throughout the winter.

(*a*) *Dry conditions.* During a hot, dry period, samples of faeces were placed on a wire sieve so that air could circulate around the pellets. The sieve was placed in front of an open window in the laboratory in such a way that the faecal pellets dried but the temperature on the sieve did not rise above 69° F. Examination of the faeces after 7 days showed that the eggs had hatched out, but that only dead larvae were found, none of these having reached the third stage.

Thus Mönnig's work (1930) was confirmed. Under conditions similar to those described above, development may be incomplete and the less resistant stages may be killed. In England such conditions will rarely occur on pastures, the protection of the grass being sufficient to prevent drying before the third stage is reached. Theoretically the most lethal conditions will occur when the temperature is just sufficiently high to bring about hatching of eggs and the rate of evaporation is high. Such conditions will occur only on portions of pasture which have no grass (see Crofton, 1948).

(*b*) *Winter conditions.* An experiment was commenced at the end of November to determine the viability of eggs after winter exposure. On 20 November five plots were chosen in grass trimmed to 4 in. in height. On each plot were placed two separate samples of freshly passed faeces, each sample weighing 10 g. Another 10 g. were used for egg counts, and 10 g. were incubated. The percentage of eggs which hatched was determined by comparing

the egg counts with the number of larvae produced after 7 days' incubation at 26° C.

After 1 month the faeces were collected from one of the plots, and the number of eggs per g. and the number of larvae developing after incubation at 26° C. were estimated. This procedure was repeated at monthly intervals, one plot being examined each month, the last one being examined on 18 April.

In December another four plots were prepared, the same technique being adopted. Three plots were laid out in January, two in February and one in March. Thus in April, five plots, which had been initially prepared at different times, were examined. Table 1 shows the time-table of the experiment, the results of which are summarized in Fig. 2.

The eggs which were exposed from November and December until April remained viable. The majority of eggs which were exposed initially during the very cold period of February and March died. Thus, although most of the eggs which remained on the plots throughout the whole winter survived, most of those exposed for the last 2 months only did not hatch. Two explanations are possible. First, the eggs when they were initially exposed were not embryonated. According to Mönnig (1930), some eggs are not resistant to dry conditions when they are not embryonated. It may be that the low temperatures killed the non-embryonated eggs only and that eggs placed on the plots in the earlier part of the experiment developed to the 'embryonated' stage before the onset of the very cold period. If this is so,

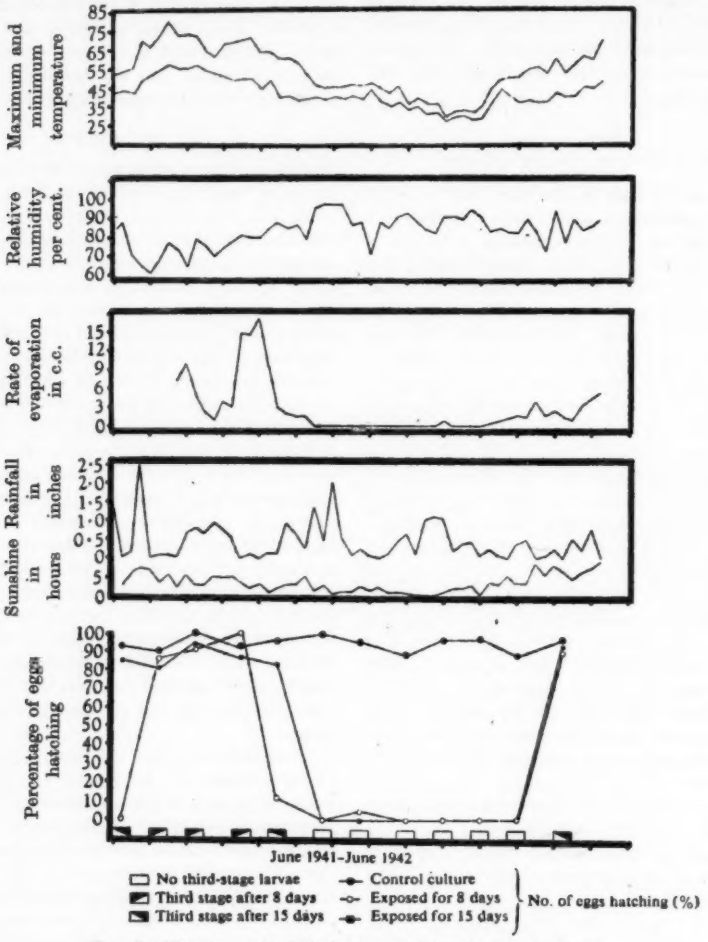

Fig. 1. Showing rate of hatching and development of eggs.

however, study of the thermograph readings shows that it is necessary to assume that development of the egg is not wholly arrested below 50° F., but that development may occur between 35 and 50° F. but only to the embryonated stage. Laboratory experiments have not confirmed this. A second explanation may be that the onset of unfavourable conditions was gradual when faeces were placed on the plots in the early part of the winter so that the eggs became slowly acclimatized. When eggs were placed on plots in February the change from the high temperature of the rectum of the rabbit to the cold conditions on the grass plots may have been so sudden that there was no time for acclimatization.

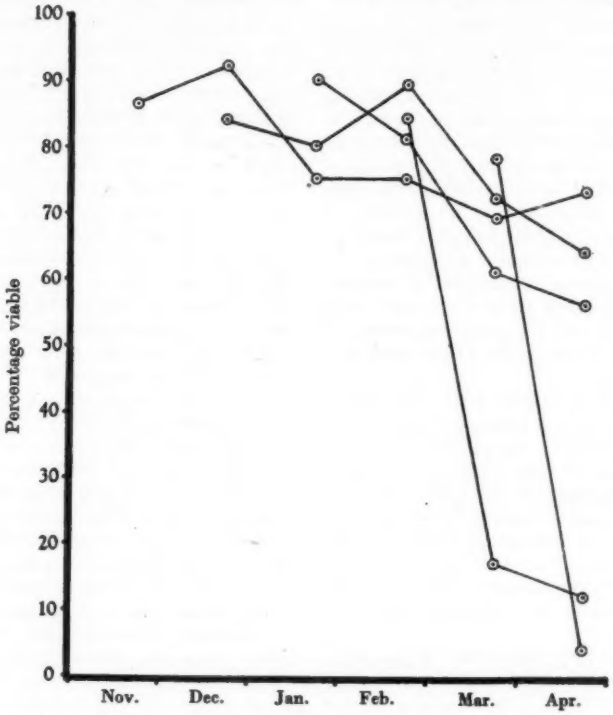

Fig. 2. The percentage of eggs hatching after exposure on grass plots during winter.

Table 1

Plots started					Sample
Nov.	Dec.	Jan.	Feb.	Mar.	collected
×	—	—	—	—	Dec.
×	×	—	—	—	Jan.
×	×	×	—	—	Feb.
×	×	×	×	—	Mar.
×	×	×	×	×	Apr.

4. ACTIVITY AND SURVIVAL OF INFECTIVE LARVAE

(i) *Daily fluctuations in the number of larvae on grass*

A plot of ground uniformly covered with grass, mainly *Festuca* spp., was used for these experiments. Each week eight plots each 4 in. square were marked out with metal strips. At the beginning of each set of observations 15,000 third-stage larvae of *Trichostrongylus retortaeformis* were pipetted on to the soil at the base of the grass blades on each plot. Twenty-four hours after the larvae had been placed on the plots, the grass on one plot was cut a quarter of an inch from the soil surface. The larvae were washed off the grass thus obtained; they were centrifuged and their number counted. Examinations were made at intervals of 24 hr., a different plot being used each time. When the eighth plot in the series was cut, another eight plots were laid out. The whole procedure was repeated throughout the months between May 1941 and June 1942. The number of larvae recovered daily was recorded throughout the whole of this period. Complete results are not given in detail,

but Figs. 3A–G show results for periods selected to represent the conditions found at different periods of the year.

Fig. 3*A. Period* 11–18 *July* 1941. During this period the temperature never fell below 50° F. and on 3 days it rose above 80° F. At the beginning of the period the rate of evaporation was high, but it fell towards the end of it. The number of larvae recovered on the first 2 days was lower than it was on the succeeding days, although the maximum temperatures were highest at the beginning. The number of larvae recovered bears a close relation to the rate of evaporation measured by the Piché evaporimeter. A high rate of evaporation corresponds with a decrease in 'catch'. A high rate of evaporation was associated with a high temperature on 11 and 12 July, but the diagram shows that an increase in temperature does not always produce an increase in the rate of evaporation. Humidity conditions must have an important influence on the amount of evaporation, but the 10 o'clock humidity bears no relation to the daily range. Increase in temperature, therefore, does not necessarily increase the number of larvae which migrate. The effect of temperature is modified by other factors, the most important of which is the rate of evaporation, which itself is a function of temperature.

Fig. 3*B. Period* 20–27 *August* 1941. The results obtained during the period resemble those which have just been described. Again the 'catch' is related to the amount of evaporation which occurs, the temperature range being smaller than in Fig. 3A, although the minimum temperature never falls below 50° F. The most interesting feature shown by this diagram is the fact that on two successive days 0·23 and 0·45 in. rainfall were recorded. There was no decrease in the number of larvae as a result.

Fig. 3*C. Period* 7–14 *October* 1941. During this period the minimum temperatures varied from 35 to 45° F. and on only 1 day did the temperature rise above 70° F. During the 8 days 1·04 in. of rain fell and the rate of evaporation was low. The diagram shows a close relationship between temperature and 'catch' and the rate of evaporation did not obviously affect the number of larvae which migrated.

Fig. 3*D. Period* 23–30 *October* 1941. During this period no temperature above 55° F. was recorded and after 24 October the temperature remained below 50° F. No evaporation was recorded during the 8 days. Between 1000 and 2000 larvae were found on the grass blades on the first 2 days when the maximum temperatures were above 50° F. On the remaining days the temperatures were lower than this, and on these days approximately 1000 larvae were recovered each day. During this period of low temperature the 'catch' was fairly constant indicating that the larvae did not migrate, the 'catch' representing the number remaining motionless from the second day.

Fig. 3*E. Period* 10–17 *December* 1941. During this period less than 1 % (150) of the total number of larvae placed on the plot were recovered each day. The maximum temperature range was 43–48° F. and the minimum temperature range 33–45° F. No evaporation occurred. Under these conditions the temperature was apparently too low for migration to occur. Comparison with Fig. 3D (23–30 October) is interesting. 23 and 24 October were warmer than the succeeding days of the latter period and migration occurred. From 25 to 30 October, however, temperature conditions were similar to those which occurred between 10 and 17 December. This fall of temperature coincided with a cessation of movement of the larvae. At the beginning of the period 10–17 December (Fig. 3E), when the temperature was low, no larvae climbed up and it may be inferred that there was no movement of larvae downwards into the soil, because no downward movement occurred on days 25–30 October when temperature conditions were similar. This inference is supported by the examinations of 'mat' and soil (Crofton, 1948). This question is again discussed below.

Fig. 3*F. Period* 25 *April to* 2 *May* 1942. During this period the maximum temperature did not exceed 50° F. for the first 5 days, but rose on the sixth day and reached 60° F. on the seventh. The rate of evaporation did not exceed 0·5 c.c./day. The relative humidity at 10 o'clock was at first low (less than 70 %), but rose to over 90 % on 1 May. The number of larvae found on the first 5 days was less than 400 per day, but on the remaining days 2000–3000 were found. The low initial 'catch' corresponds with the low temperatures and the low humidities; the increased 'catch' corresponds with the higher temperatures and humidities. Consideration of the figures over the year suggests that the apparent association between 'catch' and 10 o'clock humidity is fortuitous, but that there is a positive correlation between 'catch' and maximum temperature.

Fig. 3*G. Period* 11–18 *May* 1942. During this period the maximum temperature increased from less than 50° F. on the first day to nearly 70° F. on the seventh day. The rate of evaporation was not high and rain fell on 2 days only. An increasing 'catch' again corresponds with increasing temperature; evaporation seems to have little effect.

The 'catch' was small during the periods 26 May–18 June 1941 and 7 October 1941–1 April 1942. From 1 December 1941 to 14 March 1942, little or no migration occurred. These low larval counts occurred during periods when the temperature did not exceed 55° F. When less than 1 % of larvae were recovered, the temperature did not rise above 50° F. There is no direct relationship between the number of larvae

recovered and the rate of evaporation, rainfall or humidity at 10 o'clock, but all of these factors enter into the environment and must have had some effect.

antagonistic effects: an increase in activity of the larvae which caused them to migrate upwards and an increase in the rate of evaporation which caused a downward migration.

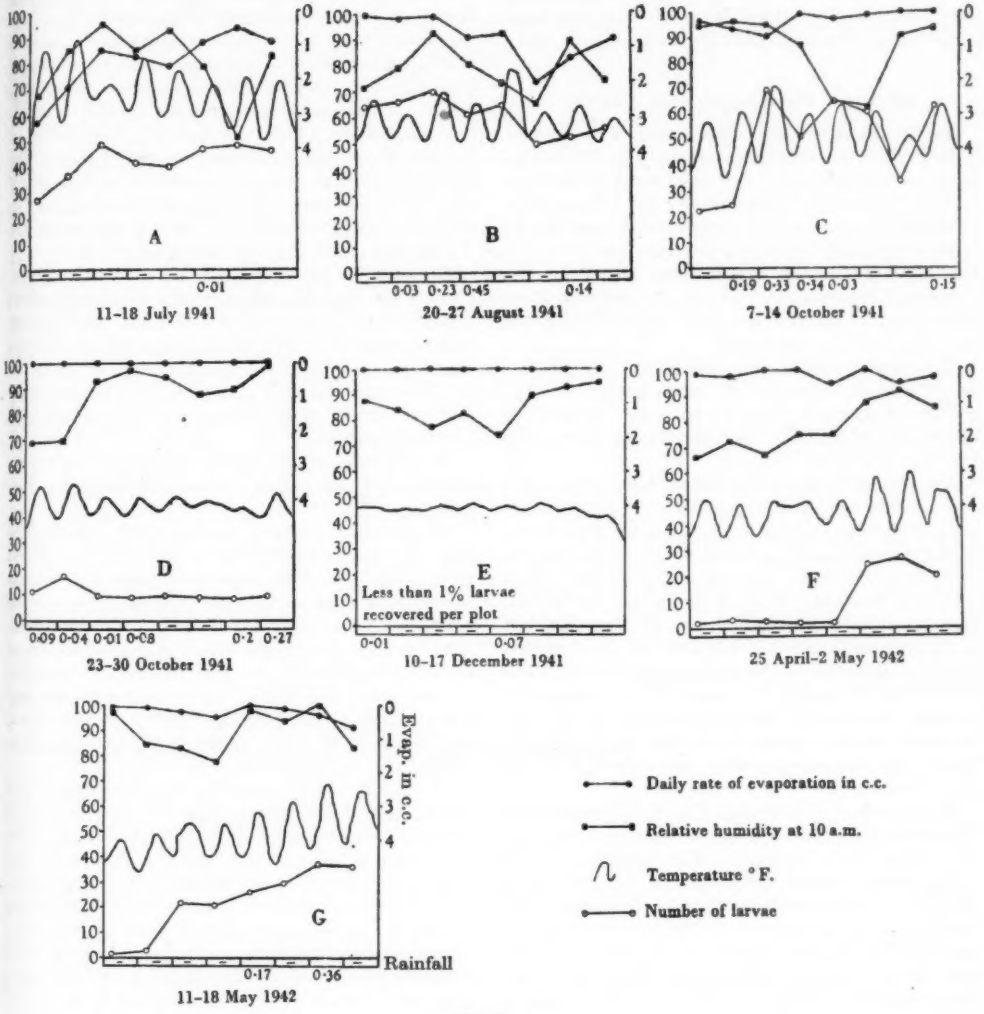

Fig. 3.

During periods of higher temperature the number of larvae migrating up the grass increased. The rise of temperature was, however, accompanied by an increase in the rate of evaporation, which is also associated with the relative humidity. It will be noted that an increase in the rate of evaporation reduced the number of larvae recovered from the plots. Rise in temperature, therefore, produced two

Usually the rainfall did not appear to affect the 'catch' directly. The increase in relative humidity and decrease in evaporation which it caused must, however, have played their part. Exceptionally the larvae were washed off the grass blades and scattered. This occurred, for example, on 22 June 1941, when 2·47 in. rain fell in 4 hr. Under the conditions of the experiment it was impossible to determine the fate of

these larvae. The number of larvae recovered from the grass blades was always very much smaller than the number originally placed on the plots. Because only the larvae normally available to the host were considered, figures for the lower portion of the blades, the 'mat' and the soil are not given here, but have been discussed in a previous paper (Crofton, 1948).

(ii) *The rate of disappearance of larvae*

Experimental method. Grass plots, 4 in. square, were marked out with metal strips. The strips were buried in the ground so that only one-quarter of an inch projected above the surface. On 1 June 1941, 4000 infective larvae of *T. retortaeformis* were placed on each of twenty-one plots. The grass was cut on one plot after 24 hr. and the number of larvae was counted. Thereafter one plot was cut and examined each week, the last plot being cut 5 months after the beginning of the experiment.

The whole procedure was repeated, a new series of twenty-one plots being started each month, until the last series was laid down in January. Records of temperature, humidity, the rate of evaporation and rainfall were kept.

Results. These are presented in Figs. 4–6.

1 *June*–12 *October* (see Fig. 4A). After 24 hr. less than 20 % of the larvae were recovered. Two weeks later the percentage had risen to 35 %. During the third week, however, more than 2 in. of rain fell in a few hours, so that the remaining plots were flooded and no more larvae were recovered. This does not suggest that the flooding killed the larvae, but that they were scattered. During the first fortnight few larvae were recovered, but since the percentage rose at each examination then presumably the low temperature was not killing the larvae rapidly but reducing the rate of migration from the soil to the grass blades.

29 *June*–9 *November* (see Fig. 4B). During the first week the number of larvae recovered was high (60–70 %). This occurred when weekly average temperature varied between 54° F. (minimum) and 72° F.(maximum). At the end of the first week the number of larvae recovered at each examination gradually decreased; so that at the end of 9 weeks only 22 % of the larvae originally placed on a plot were recovered. During the next 4 weeks the rate of evaporation was high and the number of larvae recovered was less than 5 %. Thereafter few larvae were recovered, although some were found at the end of the nineteenth week. Thus, at the end of 9 weeks nearly all the larvae had disappeared from the plots, but a few survived until November.

27 *July*–7 *December* (see Fig. 4C). For the first 5 weeks, approximately half the larvae originally placed on each plot were recovered. During the sixth and seventh weeks the number recovered fell rapidly; the rate of evaporation increasing during this period. Few larvae were recovered after the sixth week, and none was seen after the twelfth week.

24 *August*–4 *January* (see Fig. 5D). The number of larvae recovered fell rapidly from over 60 % on the first day to less than 5 % at the end of 4 weeks. Again the rapid reduction in numbers occurred during a period in which the rate of evaporation was high. No larvae were recovered after the twelfth week.

28 *September*–1 *February* (see Fig. 5E). 47 % of the larvae placed on the first plot were found at the end of 24 hr. The numbers recovered at the end of each successive week were gradually reduced until at the end of twelve weeks only 10 % were found. On 1 February small numbers were recovered.

19 *October*–8 *March* (see Fig. 5F). The disappearance of larvae during this period was gradual, some being found at the end of 20 weeks. The highest number was recovered after 1 day, but the larvae found on the blades represented only 30 % of the number placed on the soil surface. This low recovery and low rate of disappearance was associated with low temperature.

16 *November*–29 *March* (see Fig. 6G). The results were very similar to those obtained during the preceding period.

14 *December*–26 *April* (see Fig. 6H). During the first 17 weeks less than 40 larvae were recovered from each plot. The maximum temperature was never more than 45° F. and migration on to the blades must have been reduced by the low temperature. The low percentage of larvae recovered cannot therefore be ascribed to a high death-rate. Support for this view is afforded by reference to the last 3 weeks of this period during which the temperature was rising, more larvae being then recovered than during any of the preceding 17 weeks.

11 *January*–31 *May* (see Fig. 6I). Less than 1 % of larvae were recovered per plot during the first 13 weeks of this period but, during the last 7 weeks of it, more than 7 % were recovered from one plot, and more than 1 % were recovered from the other plots. Thus it must be assumed that few larvae were found during the first 4 months because the low temperatures prevented migration and that, when the temperature rose, more larvae migrated. It cannot be assumed, however, that, because little or no migration occurred until the last 7 weeks, the viability of the larvae remained unaltered by the low temperatures. The number of larvae recovered during the last 2 weeks was lower than might be expected if the larvae had been unaffected, the average weekly maximum rising above 60° F. during this time. Conditions during this period and the previous one were such that eggs would not have hatched and that no young larvae would have occurred naturally. Thus the experiments starting in December and

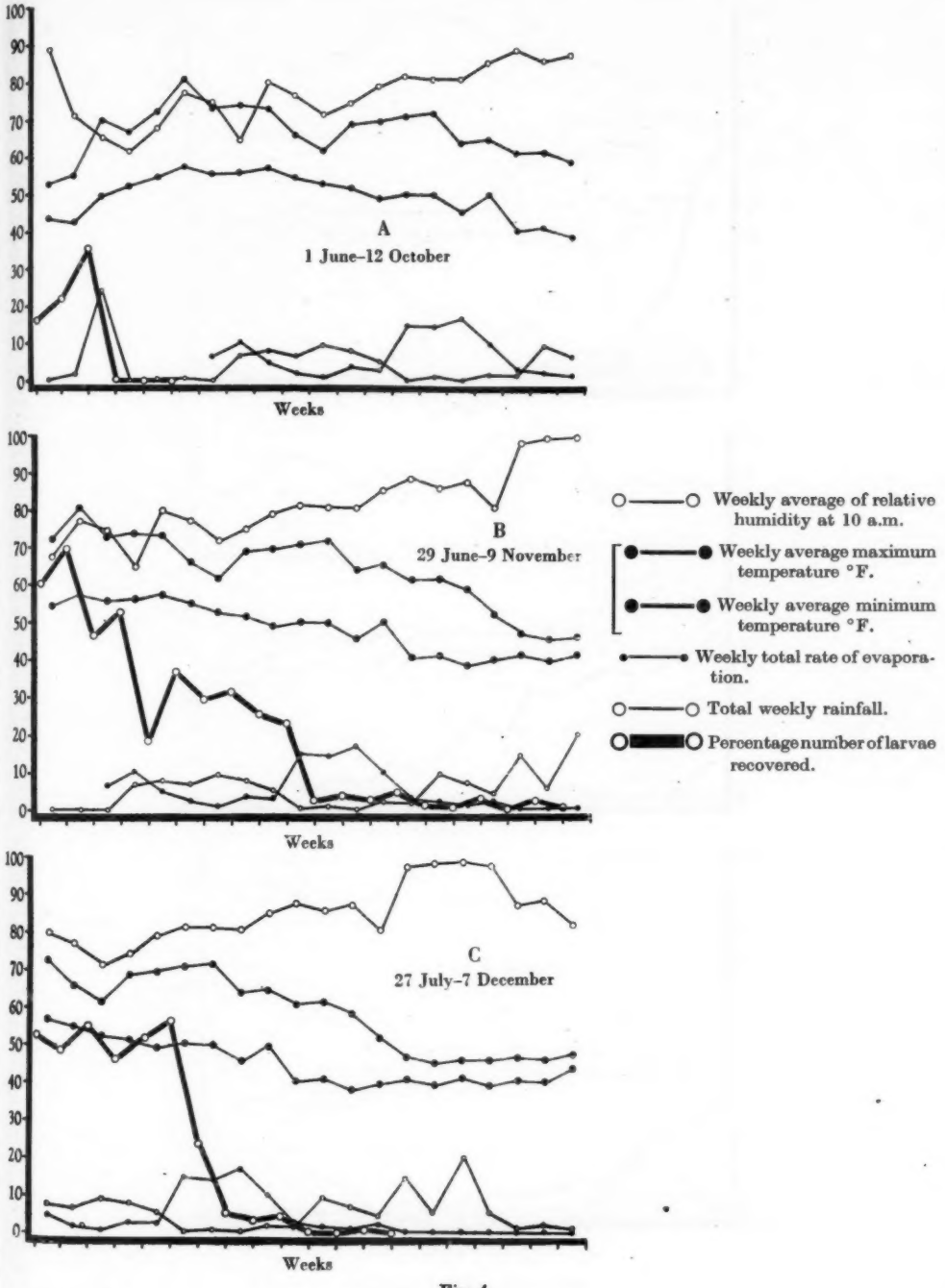
A
1 June–12 October
Weeks
B
29 June–9 November
Weeks
C
27 July–7 December
Weeks
Weekly average of relative humidity at 10 a.m.
Weekly average maximum temperature °F.
Weekly average minimum temperature °F.
Weekly total rate of evaporation.
Total weekly rainfall.
Percentage number of larvae recovered.

Fig. 4

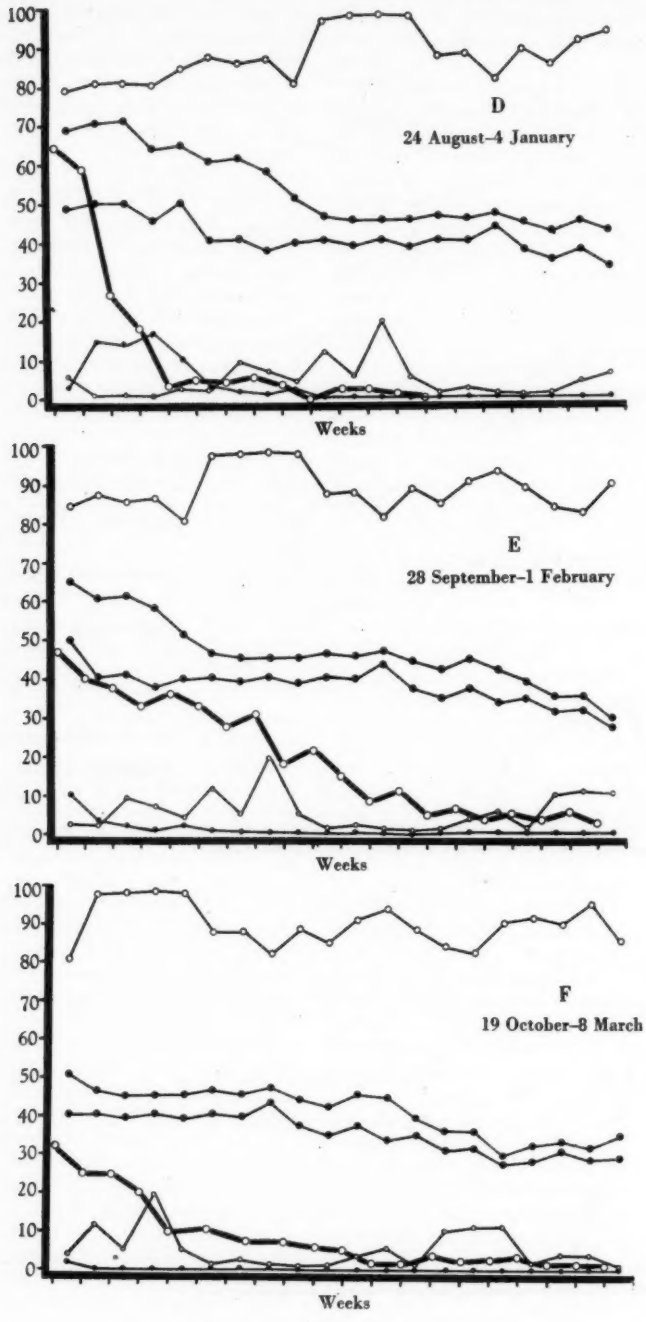

Fig. 5. (See Fig. 4 for Key.)

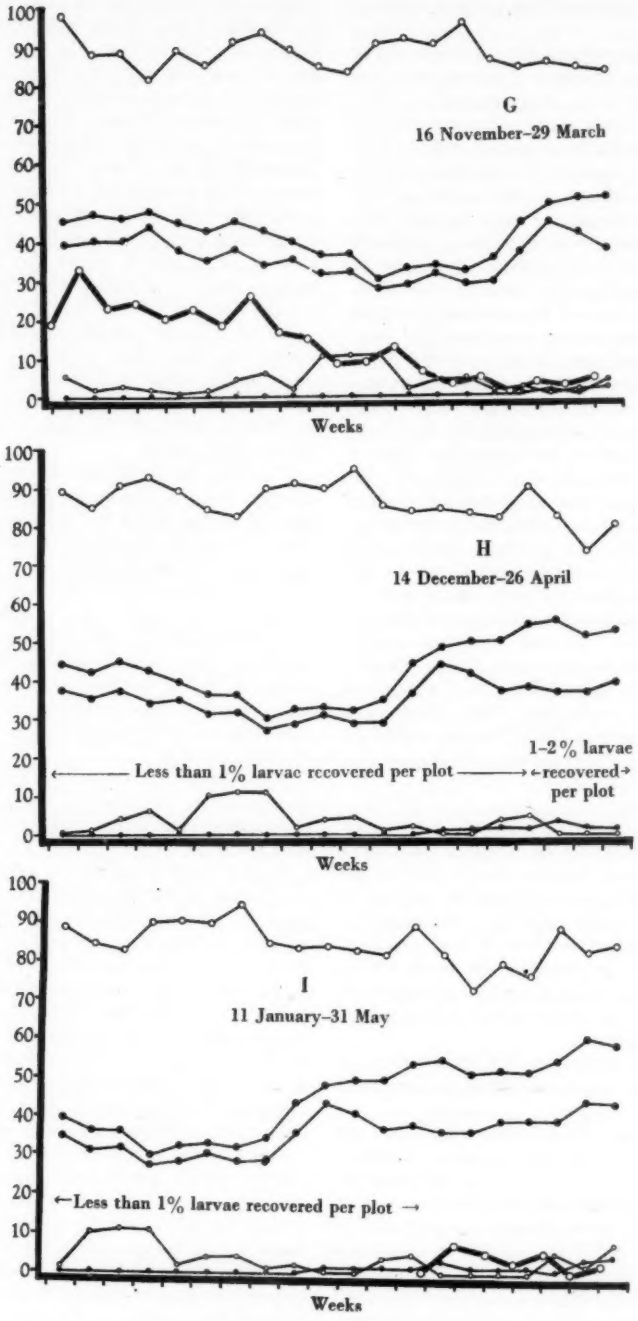

Fig. 6. (See Fig. 4 for Key.)

January were very artificial and their value is doubtful.

The experiments described above indicate that a rapid reduction in the number of larvae recovered coincided with high rates of evaporation. High temperatures were correlated with high initial 'catches', and, as might be expected, with a high death-rate, because increase in temperature, by producing greater activity, would enable a large number of larvae to migrate to more exposed positions on the grass blades when humidity conditions were suitable. Low temperature, by reducing activity, gave low 'catches' but also a low rate of disappearance. This low rate of disappearance was the result of the larvae remaining in or near the 'mat' under such conditions (see Crofton, 1948). Thus, during the summer months the rate of disappearance was high, and highest during periods of drought, when the rate of evaporation was highest. In winter, although 'catches' were low, small numbers of larvae were recovered at the end of 20 weeks. In one instance the 'catch' was greatest at the end of a long cold period, at the end of which the temperature rose. This shows that larvae may survive long periods of cold weather, during which they remain inactive. Similar conclusions have been drawn by other workers who used infective larvae of different species of Strongyloid worms (Ransom, 1906; Veglia, 1916; Theiler & Robertson, 1915; Baudet, 1925; Ortlepp, 1925; Zawadowsky & Vorobiova *et al.* 1925, 1933, 1934; Schwartz, 1925; Dikmans & Andrews, 1933; Griffiths, 1937; Taylor, 1934*a*, *b*, 1938; Baker, 1939; Shorb, 1944; Raffensperger, 1931).

5. DISCUSSION

The availability of the infective stage to the host is dependent on two main factors: the presence of the parasite in the third larval stage and the position of the infective larvae on the pastures. Obviously the rate of hatching and the survival of ova, and first- and second-stage larvae determine the number of infective larvae present, while the larvae are available to the grazing host only if they occur on that part of the herbage which is eaten.

The present work has shown that climatic factors affect development from the egg stage in four main ways.

First, conditions may be such that eggs hatch and the larvae develop to the infective stage. Development to the infective stage is mainly governed by temperature. The experiments described above show that the eggs of *T. retortaeformis* do not hatch at temperatures below 50° F. and that at temperatures above this hatching occurs and the larvae develop to the infective stage. Within the limits of the temperature range considered, the higher the temperature the more rapid was the hatching and development.

Secondly, eggs may hatch in very dry weather, but death of the larvae may occur before the infective stage is reached. The experiments described above show that this may occur on dry portions of pasture, or on land devoid of grass, but that it is not likely to occur sufficiently frequently or extensively to have any great effect on the population as a whole. Theoretically it is most likely to occur when the temperature is about 50° F., that is to say, when the rate of hatching and development is low and the rate of drying is high. Such conditions rarely occur on a pasture in England, where the presence of grass reduces the rate of evaporation from the surface of the faecal pellet (see Crofton, 1948).

Thirdly, under winter conditions no hatching occurs and the eggs may die. The experiments described above show that this occurs when eggs are deposited on the pasture during a very cold period. Normally, most of the eggs of *T. retortaeformis* survive the winter.

Fourthly, hatching may be delayed by cold conditions, but the eggs may remain viable for long periods and a rise of temperature produces apparently normal development. It has been shown that eggs passed in the autumn can survive throughout a cold winter and hatch in the spring.

The climatic conditions thus control the number of infective larvae by their influence upon the rate of hatching. They cause a large increase of the number of larvae in the warmer weather, mass hatching of eggs passed during the winter, and a period of rapid hatching of newly passed eggs during the warm summer months.

The increase of the number of infective larvae on a pasture due to the hatching of eggs and development of larvae is offset by the death of larvae which do not find a host.

Taylor (1938) has shown that there is a high initial death-rate of larvae placed on a grass plot. The experiments described above confirm this conclusion to some extent. They show that high initial death-rates occur during hot periods of the year when the larvae migrate actively; and that they occur particularly when the rate of evaporation is high. In autumn and winter the initial death-rate is lower. More important than the initial death-rate, however, is the length of time required for the complete disappearance of the larvae. In summer this period is short; but in winter it is long; and the experiment described above shows that a small number of larvae which reach the infective stage in the autumn can survive throughout the winter. Even during periods in which conditions are very lethal, some larvae remain alive for 1 month. Thus the number of infective larvae present at any time is dependent upon the rate of hatching of eggs and the rate of disappearance of larvae. During the warm period, however, eggs hatch in less than 8 days, and

possibly in 24 hr.; and some larvae from each hatching remain alive for at least 1 month. The population of infective larvae must therefore rapidly increase during the warm periods. During cold periods when no eggs are hatching, the presence of infective larvae is dependent upon the number which develop before hatching ceases and the length of time during which these survive. The experiments described above show that a small number of larvae remain alive throughout the winter and that their longevity increases during the cold periods. The conclusion can be drawn, therefore, that infective larvae of *T. retortaeformis* are present throughout the year on a pasture in England; that a large number are present during the summer, because the rapid rate of hatching more than compensates the increased death-rate of larvae caused by the higher temperatures; and that a small number are present during the winter which survive until spring. There is however no evidence to show that these larvae which survive through the winter remain infective after a long period of exposure to winter conditions. The presence of infective larvae on a pasture does not necessarily mean that they are available to the host, but the availability of those present is controlled by their activity in migrating to a position in which they may be swallowed.

The experiments described above show that the daily fluctuations of the activity of the larvae are considerable and that these fluctuations are governed by fluctuations of the temperature, humidity and the rate of evaporation. In general, a rise of temperature increases migration, but this may be offset by low humidities and high rates of evaporation which decrease migration. During cold periods little or no migration occurs, so that the small number of larvae occurring on a pasture during winter will not *normally* be available to a host. It is clear, then, that the availability of larvae at any one time is dependent not only upon the climatic factors which influence their activity at that time, but also upon the effect of past conditions on the rate of hatching and survival.

6. SUMMARY

1. Eggs and larvae of *Trichostrongylus retortaeformis* were used.

2. The rate of hatching of eggs was shown to be mainly related to temperature. From November to March, when maximum temperatures were below 50° F., there was no hatching. When maximum temperatures of 50–55° F. occurred eggs hatched on or before the fifteenth day, but never during the first 8 days. Eggs hatched in 8 days or less when maximum temperatures of 60–80° F. occurred.

3. When the rate of evaporation in the air was high, eggs still hatched and reached the infective stage, the grass blades reducing the rate of loss of moisture from the faecal pellet. Laboratory experiments show that eggs may not develop to the infective stage if the faecal pellets are on a grassless portion of the pasture. This is most likely to occur when the rate of evaporation is high and the temperature low.

4. Hatching may be delayed by cold conditions, but some eggs remain viable for long periods and they hatch when the temperature rises. Eggs passed by the host in the autumn can survive a cold winter and hatch in the spring, but eggs passed during the coldest period die.

5. During periods when the maximum temperature never exceeded 55° F., little or no migration of larvae occurred. When temperatures rose above 55° F. the number of larvae migrating increased; but rise of temperature was associated with increase in the rate of evaporation. High rates of evaporation reduced the number of larvae migrating on the grass blades.

6. Some infective larvae died soon after exposure on grass plots, but a small number survived long periods. In cold weather some larvae were still alive after 20 weeks. A high death-rate occurred in warm weather. A large proportion of the larvae died during periods in which the rate of evaporation was high; in one of these periods 95 % of the larvae were dead at the end of 4 weeks' exposure.

7. The number of larvae on grass blades of a pasture was shown to be dependent, at any time, upon the climate at that time, and upon past conditions which had influenced hatching and survival.

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SOME NEW PILICOLOUS MITES FROM SOUTH AFRICAN MAMMALS

By R. F. LAWRENCE, Ph.D., *Natal Museum, Pietermaritzburg, South Africa*

(With 8 Figures in the Text)

In March 1945 the writer had the opportunity of examining a young specimen of the South African lemur, *Otolemur crassicaudatus garnetti*, formerly known as *Galago garnetti* (Ogilby), sent into the Museum by Mr N. A. W. Macpherson from Cato Ridge near Pietermaritzburg, Natal. Fourteen Cheyletid mites were found in the fur on the back of the animal, some of them fairly active and none attached to the hairs of the body. In August 1946 a further number of mites belonging to the same family was taken from the fur of the slender mongoose, *Myonax cauui punctulatus* (Gray) captured at Mooi River. Finally, a number of larval Trombidiform mites were collected from the skin of a golden mole, *Amblysomus hottentotus longiceps* (Broom), donated by Mr R. S. Crass from Curry's Post near Pietermaritzburg.

These three parasites, all belonging to the suborder Trombidioidea, are described below.

Family CHEYLETIDAE

Genus **Cheletiella** Canestrini

Cheletiella lemuricola n.sp.

(Figs. 1–3)

Types. Five males and nine females, all mounted in Berlese medium, from *Otolemur crassicaudatus garnetti* (Ogilby), Cato Ridge, near Pietermaritzburg, Natal (June 1945).

Male. The male differing from the female in having powerful pincer-like palpi with a large triangular tooth-like projection on the inner side of the femur which doubtless, together with the large robust claw terminating the appendage, serves to hold the prey when caught. The body proportionately more slender but smaller than that of the female, and with the legs, except the fourth pair, proportionately longer and stouter. The whole animal seen from above as in Fig. 1, the two dorsal scutes large and contiguous, differing in the number and arrangement of the spines from those of the female (compare Figs. 1, 3*a*). Mouthparts seen from below (Fig. 3*b*), with a large triangular tooth nearer the outer side of the pedipalp femur and above its middle point. Penis seen through the transparent body wall, Fig. 1, large and curved.

Ventral surface with fewer spines than in the female, especially at its posterior apex, and fewer than those of the dorsal surface. Third segment of leg IV with a large broad spur-like spine on its ventral surface; this structure is completely absent in the female.

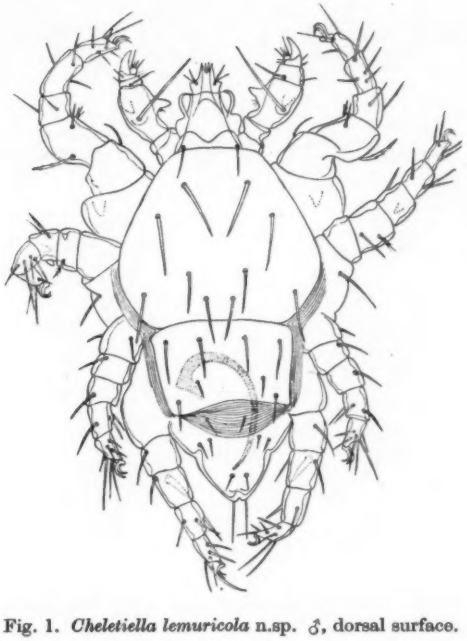

Fig. 1. *Cheletiella lemuricola* n.sp. ♂, dorsal surface.

Female. Body much larger and proportionately wider than that of the male; legs, except the fourth pair, short and less robust than those of the male (Fig. 2). Dorsal surface with two distinctly separated scutes armed with fairly broad spines as in Fig. 3*a*. These spines differing from those of the male in their larger number and pattern of distribution. Ventral surface without scutes and with spines arranged as in Fig. 2, these markedly shorter and more slender than those of the dorsal surface.

Pedipalp quite different from that of the male in lacking a large robust claw at the apex of the

segment (Figs. 2, 3*c*), the terminal segment modified for clasping hairs, its apex rounded and curved downwards and provided on the under side with a number of small finger-like teeth, the whole resembling a small clenched hand; inner surface of the penultimate segment with a rounded process, but without the sharp triangular tooth-like process of the femur of male pedipalp. The hypostome also rather differently constructed from that of the male.

Legs differing from those of the male in the latero-posterior angles of coxae I and II which have large triangular tooth-like processes, directed posteriorly.

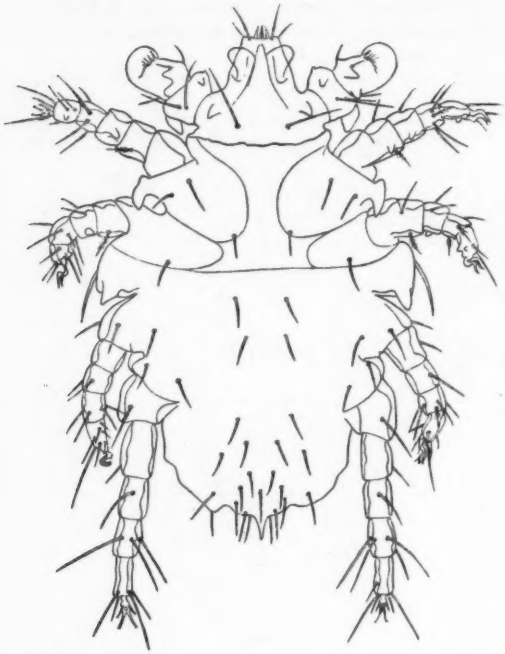

Fig. 2. *Cheletiella lemuricola* n.sp. ♀, ventral surface.

Dimensions. Male, length of body 500 μ, width 240 μ. Female, length of body 543 μ, width 348 μ.

This peculiar species differs from most hitherto described forms of Cheyletidae in the heteromorphism of the sexes. According to the generic keys of this family given by Banks (1915), and more recently by Womersley (1941), the males and females would occupy different genera. The male palpi are obviously modified for raptorial purposes and this fact and the longer and stronger legs make it probable that the male is the more active and predatory of the two sexes. The palp of the female, on the other hand, is adapted for clasping hairs; the larger body and proportionately shorter legs make it seem probable that her movements are more leisurely.

Cheletiella curvidens, n.sp.

(Figs. 4–6)

Types. One male and 7 females, from the fur of *Myonax cauui punctulatus* (Gray) (slender mongoose), Otto's Bluff, near Pietermaritzburg (August 1946).

Male. Whole body smaller and proportionately more slender than in the female. Dorsal scute in one piece, with spines arranged as in Fig. 6*b*, the spines longer and somewhat stouter than those of the ventral surface. Ventral surface and spination as in Fig. 4. Leg I longest. Pedipalps as in Fig. 4, terminating in a robust claw, the femur with a large strong pointed projection on its outer side near the base.

Female. Whole body oval in shape, rather more thick-set than in the male, the legs more robust. Dorsal scute composed of two contiguous plates, with spines arranged in a pattern as shown in Fig. 6*a*, the spines longer and stouter than those of the under surface. Ventral surface with a distinct subquadrate plate near its posterior end, this plate absent in the male.

Pedipalp quite differently constructed from that of the male, the appendage terminating in a very large powerful hooked claw, which curves downwards and is almost bent upon itself, femur (?) with a downwardly projecting triangular process.

Dimensions. Male, length of body 337 μ, width 174 μ. Female, length of body 400 μ, width 230 μ.

This species does not seem to resemble any previously described form and, as in *C. lemuricola*, there is a marked difference in the structure of the palps in the two sexes. The curved claw at the apex of the pedipalp tarsus of the female may also be an adaptation for grasping a hair by hooking the palp round it, while this structure in the male is pincer-like.

Family TROMBIDIIDAE

Sub-family LEEUWENHOEKIINAE

Genus **Leeuwenhoekia** Oud.

The specimens described below appear to be most closely related to the genus *Leeuwenhoekia*, and I propose to leave them there provisionally. They differ, however, from all species of *Leeuwenhoekia* of which I have been able to see descriptions or figures, in the peculiar shape of the hairs of the dorsal surface and those of the dorsal scute. Eyes appear to be absent, or they are so small as to be indistinguishable

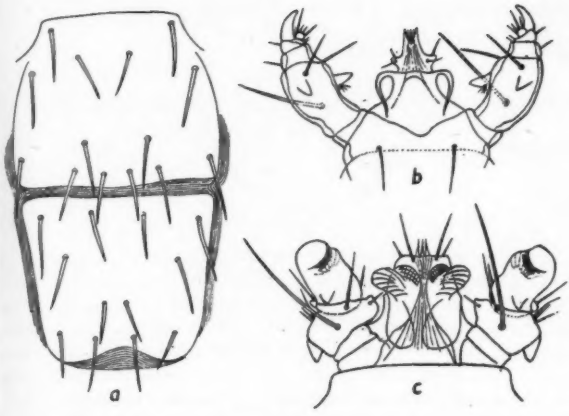

Fig. 3. *Cheletiella lemuricola* n.sp. (*a*) dorsal scutes of ♀; (*b*) pedipalps and mouthparts of ♂, ventral aspect; (*c*) pedipalps and mouthparts of ♀, dorsal aspect.

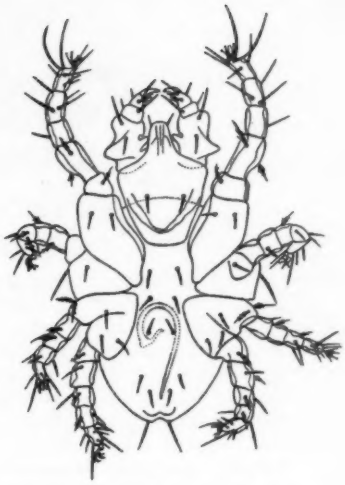

Fig. 4. *Cheletiella curvidens* n.sp. ♂, ventral surface.

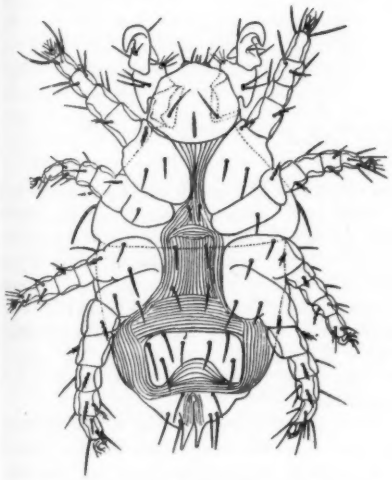

Fig. 5. *Cheletiella curvidens* n.sp. ♀, ventral surface.

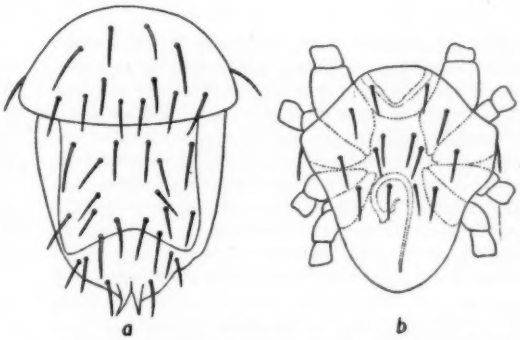

Fig. 6. *Cheletiella curvidens* n.sp. (*a*) dorsal scutes of ♀; (*b*) dorsal scute of ♂.

from other structures. A stigmatal opening between coxa I and the gnathostome, a character which, according to Womersley, distinguishes *Leeuwenhoekia* and *Hannemannia* from the other genera of the sub-family, is absent. Womersley (1945) has recently accorded the sub-family Leeuwenhoekinae full family rank.

Leeuwenhoekia womersleyi n.sp.

(Figs. 7, 8)

Types. Eight larval specimens from the golden mole, *Amblysomus hottentotus longiceps* (Broom), R. S. Crass, Curry's Post, near Pietermaritzburg (June 1945).

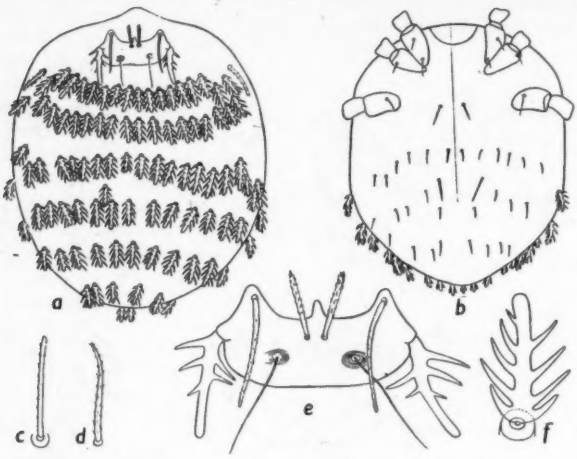

Fig. 7. *Leeuwenhoekia womersleyi* n.sp. (*a*) dorsal surface of body; (*b*) ventral surface of body; (*c*) hair of the ventral surface, enlarged; (*d*) hair from ventral surface of coxae, enlarged; (*e*) dorsal scute, enlarged; (*f*) hair of dorsal surface, enlarged.

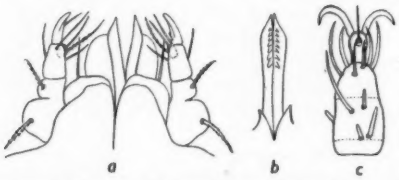

Fig. 8. *Leeuwenhoekia womersleyi* n.sp. (*a*) mouthparts from above; (*b*) chelicerae from below; (*c*) tarsus of leg II from below.

Dorsal surface. Dorsal scute as in Fig. 7 *a*, *e*, the hairs of the postero-lateral angle very large, thick, and with conspicuous pointed branches; the sensillae apparently not ciliated or only indistinctly so. A distinct conical process in the middle of the anterior margin of the scute. Body hairs behind the dorsal scute, Fig. 7 *a*, *f*, arranged in fairly regular transverse rows, the front row with 18 or 19, the second with about 18, the third with about 20, the fourth with 13 or 14, the fifth with 10, the sixth with 5 or 6. The lateral hair of the first (anterior) row on each side quite differently shaped from the remaining ones, being longer and unbranched, though provided with distinct spicules. Eyes absent or very indistinct.

Mouthparts as in Fig. 8*a* seen from above, chelicerae seen from below, Fig. 8*b*, with 6 or 7 teeth decreasing in size distally.

Ventral surface as in Fig. 7*b*, the hairs much less regular, small and inconspicuous when compared with those of the dorsum; under higher magnification (Fig. 7*c*) they appear to be of more or less even width along their whole length and truncate at their apices. Coxa I with 2, coxae II and III with 1 hair each, these tapering distally and appearing under higher magnification as in Fig. 7*d*. Apparently no stigmatal opening between coxa I and the gnathostome.

Legs. With fairly stout slightly tapering hairs provided with short fringes; trochanters I and II below with a single hair plumed only on one side; claws simple and paired, as in Fig. 8*c*.

Dimensions. Length of body, including mouthparts, 586 μ, width 456 μ.

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THE RESPIRATORY HORNS OF *PSYCHODA* PUPAE (DIPTERA: PSYCHODIDAE)

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(With 27 Figures in the Text)

INTRODUCTION

In the pupae of Diptera the prothoracic spiracle closes and the spiracular chamber acquires a connexion to a variously developed hollow cuticular outgrowth, the prothoracic or respiratory horn. The variety of form exhibited by these horns is very great, and they are extensively used as sources of diagnostic characters in the identification of dipterous pupae. De Meijere (1902) made the first systematic attempt to describe their structure in the various families, and he drew his information both from his own observations and from the scattered descriptions in the literature. Since this paper there has been little critical work on these structures, and the only horns of which we have a detailed account are those of *Simulium* (Simuliidae) (Taylor, 1902; Pulikovsky, 1927) and of a number of species of Blepharoceridae (Tonnoir, 1923, 1925). In both these families the respiratory horns differ greatly from the more generalized type, as they are modified in connexion with a life in swiftly flowing streams. Our knowledge of the more generalized type of respiratory horn is, however, very incomplete, and much of De Meijere's work could usefully be repeated.

In the course of collecting and culturing the larvae of *Psychoda* for a previous paper the writer was able to obtain the pupae of fourteen of the sixteen British species. As certain of these, notably *Ps. severini* Tonn. and *Ps. alternata* Say, are very readily cultured, the opportunity arose to reinvestigate the structure and development of the respiratory horn, and to review the variety of form seen in the horns of the British species.

MATERIAL AND METHODS

The pupae of the various species of *Psychoda* have been obtained by catching the flies in the field and setting them up to breed in culture vessels, using scalded cow dung as a medium. This method was found unsuitable, however, for the study of the development of the horn, as it was difficult to observe the larvae in the opaque medium. To overcome this a transparent medium devised by Souza Lopes (1935) was used, consisting of nine parts of nutrient agar and one part of horse-blood serum. This medium is quite transparent, and Petri dish cultures can be inspected without even removing the lid.

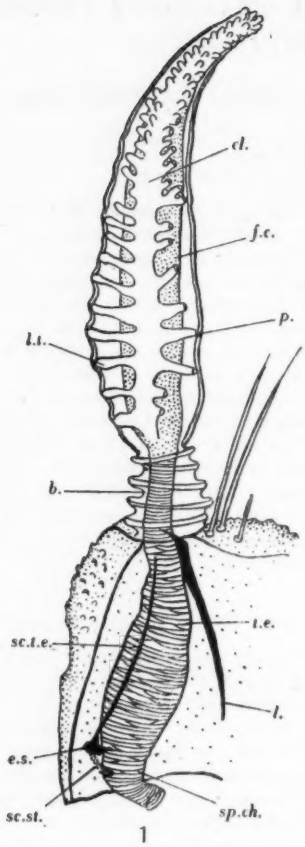
Larvae and pupae were sectioned using a modified version of the methyl benzoate-ceresin-wax technique of Espinasse (1939). The material to be cut was fixed in Carnoy, washed in 95 % alcohol, and transferred to methyl benzoate for 24 hr. followed by benzol for an hour. It was then put in the oven in a mixture of benzol and ceresin wax for ½ hr., followed by a further ½ hr. in pure ceresin wax. The latter consisted of 60° C. paraffin wax with the addition of 10 % of ceresin. This mixture gives an extremely hard wax which provides excellent support and obviates the need for double embedding. The sections were cut at 10 μ and stained in Ehrlich's haematoxylin and eosin.

THE STRUCTURE OF THE HORN

Studies on the structure of the respiratory horn were carried out on *Ps. severini* Tonn. subsp. *parthenogenetica* Tonn., as this was the only species obtainable at the local sewage works at the time the work commenced. All the major anatomical features noted were subsequently verified on *Ps. alternata* Say, the species used in the study of development. Apart from differences in the shape, and the number of pits, the horns of the two species are alike.

The horn is inserted on the anterior dorsal region of the thorax, and lies in a slight depression of the surface. On the inner side it is flanked by a thickened ridge of cuticle, and close to the base are two long and one short sensillae; more medially lies a second short sensilla.

The horn (Fig. 1) has the form of a curved tapering cylinder narrowed at the base, where there is an annulated region consisting of four or five thickened rings. Within the horn is a tube lined with chitinous felt, termed by De Meijere (1902) the felt chamber, which meets the wall of the horn at a number of thin circular regions. These De Meijere terms the pits, owing to their similarity to these structures in plant cells, and at these thin regions the air in the felt chamber is separated from the atmosphere only by a delicate wall of cuticle (Fig. 2). Much discussion has centred on the question of whether these pits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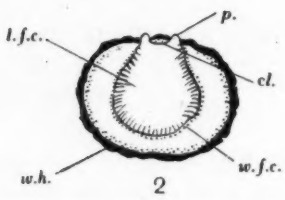
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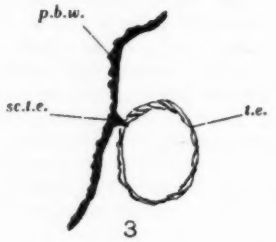
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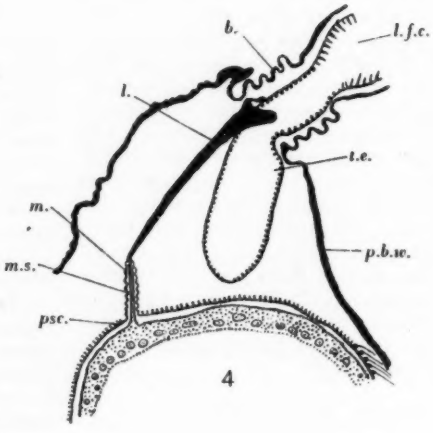
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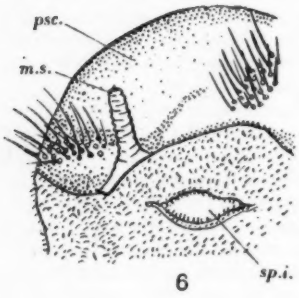
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are open or closed, and Gäbler (1935) reviews the problem and comes to the conclusion that in the higher Diptera, at least, they are open. He observed that when pupae of certain flies, such as *Drosophila fenestrarum* Fall, are placed in olive oil, it penetrates through the pits and infiltrates the tracheal system. As he recommends dipping the pupae first in absolute alcohol, a treatment calculated to increase the permeability of the pit membrane to oils, his evidence cannot be taken as conclusive, and in *Psychoda* a very definite membrane can be seen in which no trace of an opening is visible. In the basal half of the horn, the pits are regularly arranged in two rows and communicate with the felt chamber by conspicuous lateral tubes, but more apically the pits are densely crowded and lose their linear arrangement; here their lateral tubes are very short, as the wall of the felt chamber and that of the horn are closely approximated. The pits extend from the base of the horn, along the whole of the posterior face, over the apex and down the anterior face for a short distance. The chitinous felt is absent from the pits, their lateral tubes, and the area of wall enclosed between their bases.

In cross-sections of the horns of recently emerged pupae two layers of cells are visible, one being the hypodermis of the horn wall and the other the epithelium of the felt chamber. In older pupae the nuclei become indistinct and the layers tend to degenerate, whilst in mature pupae they have disappeared altogether. These features are best seen in a cross-section of the horn of *Ps. alternata* (Fig. 2), as the short lateral tubes do not tend to obscure the arrangement. The degeneration of the tissue in the horn is to be expected, as the space between its wall and that of the felt chamber is in free communication with the body cavity and therefore accessible to the moulting fluid secreted by the forming imago.

The felt chamber communicates, through the annulated base of the horn, with a large barrel-shaped chamber which has a strong and characteristic retiform thickening. This structure De Meijere (1902) termed the scar felt chamber (Narbenfilzkammer), but this term is not entirely suitable. The structure arises quite separately from the felt chamber proper, and felt is often entirely lacking, as it is here. The term tracheal extension (Miall & Hammond, 1900) is preferable, though, as Pulikovsky (1927) points out, the structure is only analogous to a trachea, as it arises in quite a different way, by a longitudinal invagination of the imaginal disk in the region separating the spiracular chamber from the horn base.

The tracheal extension is fused with the body wall on its side, the line of fusion being marked by an elongate sclerotized scar which represents the closed lips of the invagination (Fig. 3). At its lower end the tracheal extension passes imperceptibly into the spiracular chamber, and there is no sign of the diaphragm of inwardly projecting hairs separating these structures, such as has been described in *Simulium* by Taylor (1902). The elongate scar of the tracheal extension terminates in a dark sclerotized plug, the closed pupal spiracle, which De Meijere terms the outer stigmatic scar. It is connected to the spiracular chamber by a short chitinous thread, the scar strand, which is the shrunken remnant of the tube through which the larval tracheae were withdrawn. At the juncture of the scar strand with the spiracular chamber is a smaller inner stigmatic scar.

From the spiracular chamber a trachea is given off that enters the first or prothoracic spiracle of the imago. This spiracle is of the type found in the thorax of most insects, and consists of two semicircular sclerotized lips which can be closed by the contraction of a band of muscle running between them. This constitutes the closing mechanism of the imago, and Snodgrass (1935) refers to it as the 'lip' type of mechanism. An entirely different type of closing mechanism is present in mature pupae at the upper end of the tracheal extension. It consists of a closing lever (Figs. 4, 5) which is narrow basally, but broadens out apically and bifurcates into two short arms which partially encircle the tracheal extension and are attached to the horn wall by their tips. Opposite the closing lever, on the outer side of the horn, the surrounding ring of thickened cuticle is intucked. Attached to the tip of the lever is a closing muscle, which has its origin on the anterior lateral corner of the prescutum of the imago. The muscle is closely invested by a delicate cuticular sheath which is continuous with the lever at one end

Explanation of figs. 1–6

Fig. 1. The respiratory horn of *Psychoda severini* Tonn. subsp. *parthenogenetica* Tonn., as seen in the pupal exuviae. Fig. 2. Transverse section of horn of *Ps. alternata* Say. Fig. 3. Transverse section of tracheal extension and adjacent body wall of *Ps. severini*. Fig. 4. Horizontal longitudinal section of pupa of above through the closing mechanism. Fig. 5. The closing lever. Fig. 6. Surface view of thorax of a recently emerged fly, to show the adherent muscle sheath. All figures × 308.

b. base of horn; *cl.* clear area of felt chamber where felt is lacking; *e.s.* external stigmatic scar; *f.c.* felt chamber; *l.* closing lever; *l.f.c.* lumen of felt chamber; *l.t.* lateral tubes; *m.* closing muscle; *m.s.* closing muscle sheath; *p.* pits; *p.b.w.* pupal body wall; *psc.* prescutum; *sc.st.* scar strand; *sp.ch.* spiracular chamber; *sp.i.* prothoracic spiracle of imago; *sc.t.e.* scar of tracheal extension; *t.e.* tracheal extension; *w.f.c.* wall of felt chamber; *w.h.* wall of horn.

and the imaginal cuticle at the other. When the muscle is in a contracted condition the sheath is wrinkled, but in a relaxed condition it fits it closely and evenly. This sheath probably serves to protect the muscle from the histolysing activity of the moulting fluid which circulates between the pupal and imaginal cuticle. But for this protection the muscle would probably suffer the same fate as the hypodermis and felt chamber epithelium of the horn. The delicate nature of the sheath in no way impedes the contraction of the muscle, which is able to remain operative right up to the moment before eclosion.

In the belief that the imago should show some trace of this sheath, since it is continuous with the imaginal cuticle, a number of recently emerged flies were examined. It was found that the sheath breaks at the point where it joins the lever, and comes away entire. It can be seen as a delicate tube of colourless cuticle projecting from the anterior lateral corner of the prescutum (Fig. 6), but as the fly dries the sheath shrivels and breaks off.

The cuticle of the pupa is too opaque to permit observation of the movements of the closing mechanism, but some idea of its action may be conjectured by a consideration of its parts. The lever is inclined at an angle of 45° at rest, but on contraction of the muscle, would tend to be pulled into a more vertical position. This would result in the upper flattened portion of the lever pressing the tracheal extension against the ridge of thickened cuticle on the opposite side (Fig. 4). The tracheal extension has its retiform thickening less strongly developed in this region, which would facilitate the compression. Snodgrass (1935) refers to this type of closing mechanism, where closure is effected by the compression of a tube, as the 'pinchcock' type.

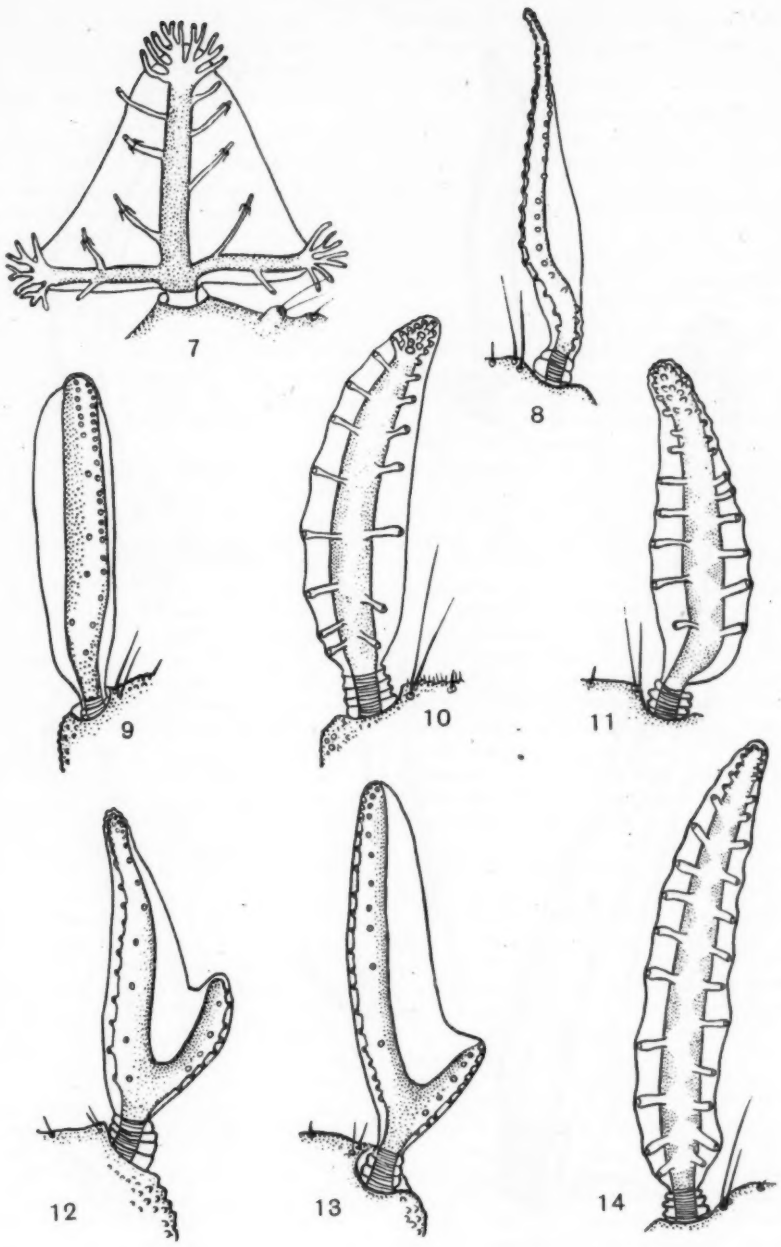
Though the literature on closing mechanism is extensive, there is only one account of such a structure connected with the respiratory horn of a dipterous pupa. In *Simulium* (Pulikovsky, 1927) a closing mechanism is present at the upper end of the tracheal extension, in the same position as in *Psychoda*, but it is of a totally different construction, and operates, not by compressing the tracheal extension, but by inflecting a valve over the opening at the base of the horn. It is known that spiracular closing mechanisms serve to limit water loss through the spiracles (Mellanby, 1934; Wigglesworth & Gillett, 1936), and insects usually keep their spiracles closed, opening them only sufficiently to supply their oxygen requirements. Though, as Pulikovsky (1927) points out, the closing mechanism in the pupa is only analogous to that in the adult, as it closes, not a trachea, but the tracheal extension, a similar water-conserving function may provisionally be ascribed to it. In *Simulium*, Pulikovsky (1927) regards it as an adaptation to life in mountain streams where the water flow may cease altogether at times. Closing mechanisms connected with the respiratory horns of dipterous pupae are probably very much more common than the dearth of literature on the subject would indicate, and a survey of their occurrence in terrestrial, marginal and aquatic forms would yield results of great interest.

THE VARIETY OF FORM SEEN IN THE RESPIRATORY HORNS OF THE BRITISH SPECIES OF *PSYCHODA*

Tonnoir (1940) lists sixteen species and two subspecies of *Psychoda* in Britain, and the respiratory horns of fourteen of these are known. That of *Ps. severini* has already been described; its specific characters are that it has an unbranched felt chamber and between ten and twelve pairs of conspicuous lateral tubes in the basal half. The horns of *Ps. spreta* Tonn., *Ps. albipennis* Zett., and *Ps. trinodulosa* Tonn. conform to this type. In *Ps. spreta* (Fig. 14) there are between ten and twelve pairs of lateral tubes, but the region of irregular, non-linear pitting is restricted to the tip of the horn. In *Ps. albipennis* (Fig. 10) there are only seven or eight pairs of prominent lateral tubes, and the horn is shorter and broader, whilst *Ps. trinodulosa* (Fig. 11) has a similar horn but the area of non-linear pitting is greater.

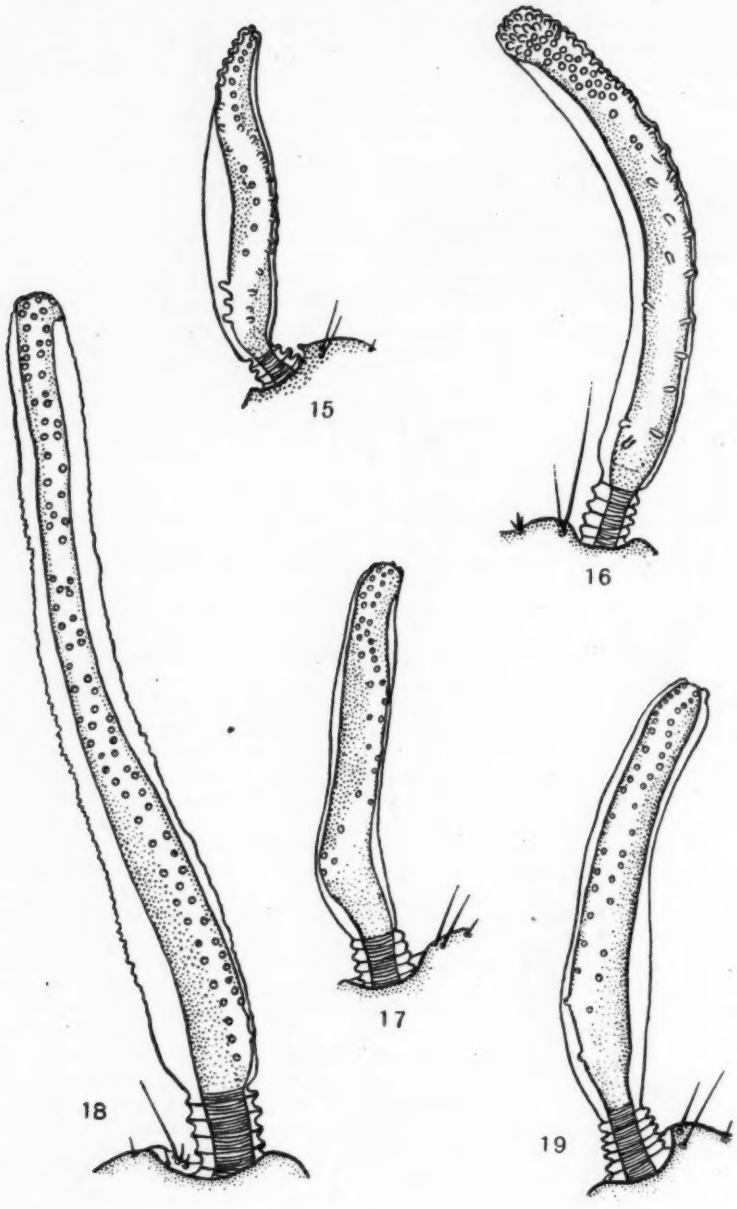
In the species *Ps. alternata* Say, *Ps. surcoufi* Tonn., *Ps. cinerea* Banks, *Ps. gemina* Eaton, *Ps. grisescens* Tonn., *Ps. setigera* Tonn. and *Ps. lobata* Tonn. the felt chamber is closely applied to the horn wall along its whole length, so that the long lateral tubes present in the previous species are not here apparent. *Ps. alternata* (Fig. 18) has a long horn with a wrinkled outer wall and bears numerous pits; as many as fifty-five may be present in each row. *Ps. surcoufi* (Fig. 16) has a shorter, more curved horn in which the basal two-thirds bear ten or eleven pairs of pits, whilst in the apical third they lose all sign of linear arrangement and spread over the whole of the posterior face. *Ps. cinerea* (Fig. 19) and *Ps. gemina* (Fig. 17) have very similar horns, which are short and blunt and bear twenty to twenty-five pairs of pits. *Ps. lobata* (Fig. 9) has a short, straight horn with a rounded apex and has twenty to twenty-five pairs of pits. The horn of *Ps. grisescens* (Fig. 15) narrows in the apical third and bears twenty to twenty-five pairs of pits, whilst *Ps. setigera* (Fig. 8) has the apical narrow region of the horn more prolonged and bears between thirty and forty pairs of pits.

A departure from the condition in the species so far described is seen in *Ps. phalaenoides* L. subsp. *elongata* Tonn. (Fig. 13) and *Ps. crassipenis* Tonn. (Fig. 12). In these species the felt chamber bifurcates at the base of the horn, giving off a short lateral branch. This bears between six and ten pairs of pits,



Figs. 7–14

Fig. 7. Respiratory horn of *Psychoda brevicornis* Tonn. Fig. 8. *Ps. setigera* Tonn. Fig. 9. *Ps. lobata* Tonn. Fig. 10. *Ps. albipennis* Zett. Fig. 11. *Ps. trinodulosa* Tonn. Fig. 12. *Ps. crassipenis* Tonn. Fig. 13. *Ps. phalaenoides* L. subsp. *elongata* Tonn. Fig. 14. *Ps. spreta* Tonn. All × 308.



Figs. 15–19

Fig. 15. Respiratory horn of *Psychoda grisescens* Tonn. Fig. 16. *Ps. surcoufi* Tonn. Fig. 17. *Ps. gemina* Eaton. Fig. 18. *Ps. alternata* Say. Fig. 19. *Ps. cinerea* Banks. All × 308.

whilst the main branch bears between sixteen and eighteen pairs. The two horns differ only in that the horn wall is more sharply incised between the main and lateral branches of the felt chamber in *Ps. crassipenis*.

The greatest departure from the condition in *Ps. severini* is seen in the horn of *Ps. brevicornis* Tonn. (Fig. 7). It is flattened and triangular and the felt chamber divides into three at the base, one branch running to each corner of the triangle. Here they divide into a number of fine tubes which penetrate the horn wall and project as clusters of air-containing filaments which are more numerous apically (twelve to twenty) than basally (eight to twelve). From the three main branches of the felt chamber other tubes arise farther back, which project from the surface of the horn in an irregular fashion. The tracheal extension and closing lever are as in the other species

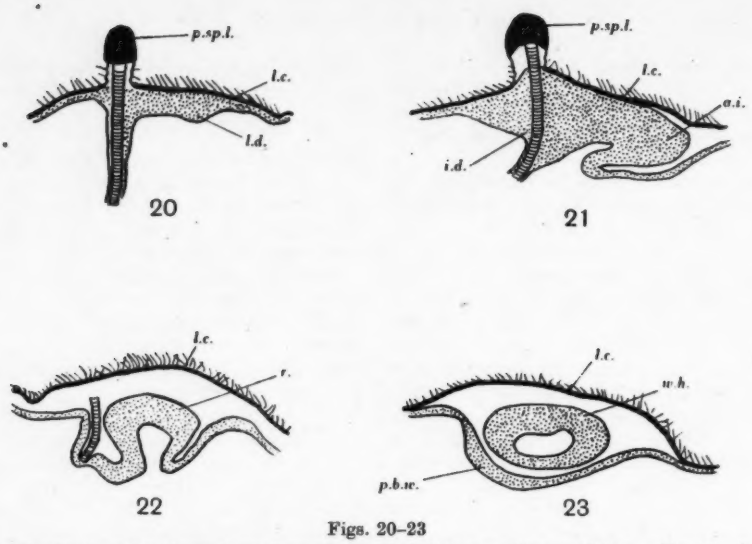
The pupa of *Ps. brevicornis* differs from that of the other species in its behaviour as well as in the structure of the horn. These other species occur in a variety of media such as dung and mud, and details of their larval habitats have been described in a previous paper (Satchell, 1947). The pupae normally rest upright in the media with only the horns exposed. That of *Ps. brevicornis*, however, lies flat on the surface of the deposit of cow dung, and, after emergence from the larva, contrives to spread the larval skin over the whole of the dorsal surface, like a closely fitting blanket, leaving only the horns projecting from the anterior end. Though the end-result, the pupa with the covering larval exuviae, has been repeatedly seen, the actual process of investment has not. Feuerborn (1923) has described the larva of *Ps. eximia*, which shows certain features, notably the semicircular eleventh segment carrying a minute siphon at the tip, in common with that of *Ps. brevicornis* (Satchell, 1947). The pupa of *Ps. eximia* is said to pupate actually within the larval skin, so that the respiratory horns project from the split in the prothorax. This is what *Ps. brevicornis*, at first sight, appears to do, but further observation shows that the entire skin, intact except for the split in the prothorax, can be lifted off the pupa and turned to one side. The retention of the last larval skin by the pupa occurs in other Nematocera, and Saunders (1924) reviews the known cases of it. The Scatopsinae (Bibionidae) are like *Ps. eximia* in having only the pupal horns projecting, whilst *Dactylolabis* and *Cylindrotoma* (Tipulidae) achieve a more complete emergence. *Forcipomyia* and *Atrichopogon* (Ceratopogonidae) retain the skin only on the last four or five abdominal segments. What purpose the retention of the last larval skin serves is unknown, but as the pupa of *Psychoda brevicornis* lies exposed on the surface of the dung, unlike other species which achieve a partial protection by being almost wholly immersed in the media, it is possible that the skin serves as a protection against desiccation.

THE DEVELOPMENT OF THE HORN

In mature larvae about to undergo pupation the respiratory horns are visible through the larval cuticle and can be seen almost to encircle the prothorax, their bases being dorsal in position, and their tips ventral. The tracheal extension lies immediately behind and parallel with the horn, its upper end communicating with the horn base. The horn is thus bent through an angle of 180° at the annulated basal region. A clear appreciation of the position in which these structures lie in the mature larva is essential for a correct understanding of their development.

The horns develop from paired imaginal disks which appear in the last larval instar in connexion with the prothoracic spiracles. Each disk is oval in shape, its long axis lying parallel with the body, and it is penetrated, on its posterior margin, by the trachea from the prothoracic spiracle (Fig. 20). The disk thickens and invaginates along its anterior margin forming a protruding lip of tissue (Fig. 21), which becomes converted into a hollow ridge, partially by upward growth of the lip, and partially by an intucking of tissue behind it. As a result of these movements the trachea from the prothoracic spiracle has now to enter the groove lying behind the ridge before it can penetrate its posterior wall (Fig. 22). From the lower end of the hollow ridge of tissue the horn grows out as a tubular process which extends ventrally, pushing between the larval cuticle and the pupal body wall (Fig. 23). The lumen of the tube is in communication with the body cavity of the developing pupa.

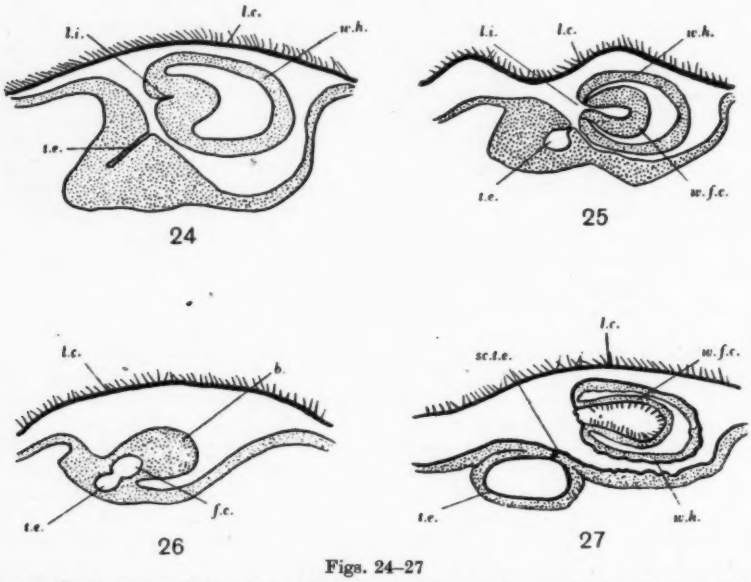
A second invagination now forms almost parallel with the first, and extends dorsally from the prothoracic spiracular trachea towards the upper edge of the disk. The region of the invagination surrounding the trachea is destined to form the spiracular chamber, and the more dorsal region will form the tracheal extension. Along the posterior face of the lengthening horn the tissue becomes very much thickened and commences to invaginate, forming a tube within a tube (Fig. 24); the outer tube becomes the horn wall and the inner tube the felt chamber. The upper part of the second invagination, destined to become the tracheal extension, has, so far, consisted of a narrow elongate slit (Fig. 24); the lips of this slit now fuse and the cavity widens out forming the lumen of the tracheal extension (Fig. 25). At this stage the horn with its contained felt chamber, and the tracheal extension, are lying side by side, the upper end of the latter lying close against the base of the horn. The cavities of the tracheal extension and the felt chamber now grow out for a short distance dorsally and meet in the region that will form the annulated base of the horn (Fig. 26).



Figs. 20–23

Fig. 20. Longitudinal section through prothoracic imaginal disk in resting condition. Fig. 21. Longitudinal section of disk showing formation of anterior fold. Fig. 22. Longitudinal section of disk showing conversion of fold into a hollow ridge. Fig. 23. Longitudinal section just below imaginal disk showing horn pushing between larval cuticle and pupal body wall. All × 308.

a.i. anterior invagination of imaginal disk; *i.d.* imaginal disk; *l.c.* larval cuticle; *p.sp.l.* larval prothoracic spiracle; *r.* hollow ridge of tissue. Other letters as in Figs. 1–6.



Figs. 24–27

Fig. 24. Longitudinal section below base of horn showing early stage in formation of felt chamber. Fig. 25. Longitudinal section in same position as above, showing later stage in formation of felt chamber. Fig. 26. Longitudinal section at base of horn showing fusion of felt chamber and tracheal extension. Fig. 27. Longitudinal section below base of horn in a fully formed pupa, just prior to its emergence. All × 308.

l.i. longitudinal invagination of horn wall. Other letters as before.

The horn is now almost complete. The wall of the horn secretes its wrinkled outer layer and the epithelium of the felt chamber secretes its lining layer of chitinous felt. The site of the longitudinal invagination that formed the felt chamber remains for some time as a groove, but becomes obliterated by the formation of pits along it, which arise as outgrowths from the felt chamber. The epithelium of the tracheal extension secretes its retiform lining layer (Fig. 27). The larval tracheae are withdrawn from the pupal prothoracic spiracle which then closes, forming the outer stigmatic scar. The short tubular region, lying between the spiracular chamber and the spiracle, contracts to form the scar strand, and the opening into the spiracular chamber closes to form the inner stigmatic scar. As soon as the pupa has freed itself of the investing larval skin the horn rotates through 180° and takes up its anteriorly projecting position.

The closing apparatus does not form until about half-way through pupal life. The closing lever arises in the hypodermis of the dorsum of the forming imago, and as the hypodermis retracts away from the pupal cuticle, the lever is withdrawn from it. As the new cuticle is laid down, a thin cuticular sheath is secreted around the closing muscle.

DISCUSSION

It is of interest to compare the account of the development outlined above with that of the two other species of Nematocera for which it is known, *Chaoborus* (*Corethra*) *plumicornis* Fab. (Culicidae) (Weismann, 1866) and *Simulium* (Simuliidae) (Taylor, 1902; Pulikovsky, 1927). In *Chaoborus* the horn originates precisely as in *Psychoda*, from a fold on the anterior margin of the prothoracic imaginal disk, but the felt chamber arises in quite a different way. The horn at first becomes almost solid by proliferation of cells from the wall, and a split then delimits a central core from the surrounding wall. Within this core a cavity arises by the formation of a second split, which widens out to form the cavity of the felt chamber. The process is thus very different from the longitudinal invagination seen in *Psychoda*. Weismann supposes that primitively the horn was formed by a protrusion of tracheal epithelium pushing into a protrusion of the hypodermis, and that the state of affairs in *Chaoborus* is merely a complication of this process. There does not appear, however, to be any evidence for this assumption in the case of *Psychoda*, as the wall of the felt chamber arises by an invagination of the horn wall. Weismann's concept of two cell layers, one tracheal and one hypodermal, does not seem to be applicable, as the entire horn, including the felt chamber, arises from the tissue of the imaginal disk. As this is penetrated by the prothoracic trachea, the possibility exists that some cells of the tracheal epithelium enter into its composition, but there is no distinction between the two cell layers at any stage.

The formation of the tracheal extension was not observed by Weismann, but Taylor (1902) has described it in *Simulium*, and Pulikovsky (1927) does not really add anything to his account. Both workers are agreed that it forms by an invagination of the hypodermis and that it subsequently acquires a connexion to the horn base. There is nothing in the two accounts of the development to suggest that two discrete cell layers are concerned in the formation of the horn, but as no felt chamber is present in *Simulium*, this is, perhaps, no criticism of Weismann's proposition. It would be of interest to know how the felt chamber arises in other genera.

There is no agreement as to the correct term that should be applied to the type of respiratory organ that the pupal horn represents. The problem is complicated by the fact that in some aquatic dipterous pupae the horns have been replaced by respiratory organs of a totally different type. In certain species of *Chironomus*, for example, *Ch. dorsalis* Meig., true tracheal gills are present in the form of tufts of filaments each of which may contain several fine tracheal branches (Miall & Hammond, 1900). In *Simulium* the pupal respiratory organ consists of a number of tubes, the thickened walls of which contain air-filled spaces which ultimately communicate with the tracheal extension. Taylor (1902) discusses the various terms that have been applied to this organ. Von Siebold (1846) had called them tracheal gills, but Taylor rejects this term as no tracheae are present. Likewise, the term tube gills given by Vogler (1900) is unsuitable, since the air is not contained within the lumen of the tube. He therefore proposes the term cuticular gill for this type of respiratory organ. These terms are all unsuitable for pupal horns in general, however, as the majority of dipterous pupae are terrestrial rather than aquatic, and their respiratory organs cannot be regarded as some sort of gill.

De Meijere (1902) proposes the term pitted stigma (Tüpfelstigma) for the respiratory horns of dipterous pupae, but this does not appear to be a legitimate use of the term stigma. In *Psychoda* the respiratory horn is neither the site of an ectodermal intucking giving rise to a trachea, nor does it have an open communication with the atmosphere. This term is therefore equally unsuitable. The variety of structure present, even amongst those pupae possessing a simple unbranched tubular horn is, however, so great, and our knowledge of this structure is so limited, that it is clearly premature to attempt to name and classify them. The horn of the chironomid *Tanypus* is clearly of a different type to that seen in *Psychoda* as no true pits are present, their place being taken by a terminal sieve-like plate bearing groups

of dot-like markings. Equally different is the respiratory horn of the Blepharoceridae, where, according to Tonnoir (1925), a series of superimposed lamellae serve to hold a bubble of air against an opening in the tracheal extension. Studies in the development of these structures are needed before it is possible to say what relation they bear to the type of horn seen in *Psychoda*. For this reason it seems wisest to retain the term respiratory horn for all these respiratory organs because it has no precise meaning and therefore defines no particular type of structural entity.

SUMMARY

1. The respiratory horn of *Psychoda severini* Tonn. subsp. *parthenogenetica* Tonn. is described. Within the tubular horn lies the felt chamber, which meets the horn wall at a number of thin circular regions, the pits, and communicates, through the annulated base of the horn, with the tracheal extension. This has a characteristic retiform thickening and is continuous, at the lower end, with the spiracular chamber, from which a trachea runs to the prothoracic spiracle of the imago.

2. At the upper end of the tracheal extension lies the closing mechanism consisting of a lever, and a muscle surrounded by a cuticular sheath. It is suggested that the mechanism functions by compressing the tracheal extension, and that the sheath serves to protect the muscle from the action of the moulting fluid.

3. The respiratory horns of fourteen of the British species of *Psychoda* are described, and it is shown that *Ps. spreta* Tonn., *Ps. albipennis* Zett. and *Ps. trinodulosa* Tonn. are like *Ps. severini* in possessing long lateral tubes in at least the basal half of the horn, whereas *Ps. alternata* Say, *Ps. surcoufi* Tonn., *Ps. cinerea* Banks, *Ps. gemina* Eaton, *Ps. lobata* Tonn., *Ps. grisescens* Tonn. and *Ps. setigera* Tonn. have only short lateral tubes. The two species *Ps. phalaenoides* L. subsp. *elongata* Tonn. and *Ps. crassipenis* Tonn. are alike in having a two-branched felt chamber, and *Ps. brevicornis* Tonn. has a flat triangular horn with a three-branched felt chamber and a cluster of air-containing tubes at each corner.

4. An account is given of the development of the respiratory horn of *Ps. alternata* Say. The horn arises as an outgrowth from the larval prothoracic imaginal disk, and the felt chamber forms by an invagination of its posterior wall. The tracheal extension arises as an invagination of the disk and subsequently acquires a connexion to the felt chamber.

5. The development is compared with that of *Chaoborus plumicornis*, and it is concluded that Weismann's theory of the origin of the horn from two distinct cell layers is not applicable to *Psychoda*.

6. The various terms that have been applied to the respiratory horn are discussed, and the conclusion is reached that though none of them is satisfactory, it is premature to devise a new scheme of nomenclature.

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ON THE EGG OF THE TICK, *IXODES RICINUS* L.

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(With 12 Figures in the Text)

CONTENTS

	PAGE		PAGE
1. Introduction	53	5. Development and hatching	57
2. Materials and methods	53	6. Summary	59
3. The egg—general features	53	References	60
4. The membranes of the egg	56		

1. INTRODUCTION

The eggs of the tick, *Ixodes ricinus* L., are not common objects in the field as they are deposited in crevices in moist situations at the bases of rush, bracken, coarse grasses and other 'mat-forming' herbage, but indications of the sites of oviposition are given by the occurrence of large numbers of larvae, which are found on vegetation during the process of 'blanket-dragging' (Milne, 1943; Edwards & Arthur, 1946*a*). Despite the fact that Nuttall (1911) and earlier workers have described the process of copulation and oviposition, very little seems to be known of the actual structure of the egg and the hatching process thereof. Regarding this latter process Nuttall (1911) writes: 'Hatching of the eggs.—A variable period of time elapses between oviposition and the hatching out of the eggs. According to Chabrier (1807) the larvae issue from the eggs after two to three weeks.' Other writers, incidentally, have recorded longer periods, thus Kossel, Schütz, Weber & Miessner (1903) place it at about 6 weeks, Bertkau (1881) at 7 weeks, Wheler (1899) at 8 weeks, Meek & Smith (cited by Wheler) at 22 weeks. The only recent additional data on the process of hatching are given by McLeod (1932), who states that the 'hatching of the larva occurred by a longitudinal rupture of the egg along one side. When newly hatched the larvae were comparatively inactive and remained for several days near the remains of the egg cluster.' Two recent notes (Arthur, 1945; Campbell, 1946) are discussed later.

One of the primary aims in this paper is to elucidate certain points of the morphology of the egg of *I. ricinus* and to describe in greater detail the visible development in living eggs and the hatching therefrom. Further, such a study is a necessary prerequisite to work on acaricidal action for the control of the tick under field conditions which is now proceeding in South Wales (Edwards & Arthur, 1946*b*).

2. MATERIALS AND METHODS

The material of *I. ricinus* employed in the present studies was obtained from dairy cows in the Margam District of Glamorgan, south Wales, through the courtesy of Mr Robert David.

Dr A. D. Lees, Unit of Insect Physiology, Agricultural Research Council, very kindly forwarded me material of *I. canisuga* Johnston, 1849, and specimens of *I. hexagonus* Leach, 1815 were obtained from a vixen trapped at Rhydyfelin, Pontypridd, through Mr Simeon, of the War Agricultural Executive Committee of Glamorganshire. To allow for maximum engorgement by the ticks only those removed from the host with a minimum of disturbance were considered as egg-laying females for the purpose of the experiment. Gorged females were kept, each in a gauze cage, enclosed within specimen tubes containing about 1½ in. of the controlling water-sulphuric acid mixture (Wilson, 1921) which maintained the humidity at 95%. Alternatively, females were confined in Petri dishes, the bases of which were covered with wet cotton-wool, over which a moistened sheet of filter paper was placed. The experiment was undertaken at room temperatures (16–23° C.) and the phases of development were examined in living eggs.

3. THE EGG—GENERAL FEATURES

The freshly laid eggs of *I. ricinus* are brown, in contrast with the lighter coloration of those of *I. canisuga* and *I. hexagonus*. This colour of *I. ricinus* eggs is due to the thin brown shell covering the colourless internal mass, which in large measure is of a globular structure (Fig. 3) with a *p*H range of 5·5–6·5. The nucleus lies embedded in this mass, surrounded by the cytoplasm with its ramifications enclosing the aforementioned globules. Typically, the freshly laid egg of *I. ricinus* is oval (Fig. 1), but deposition in

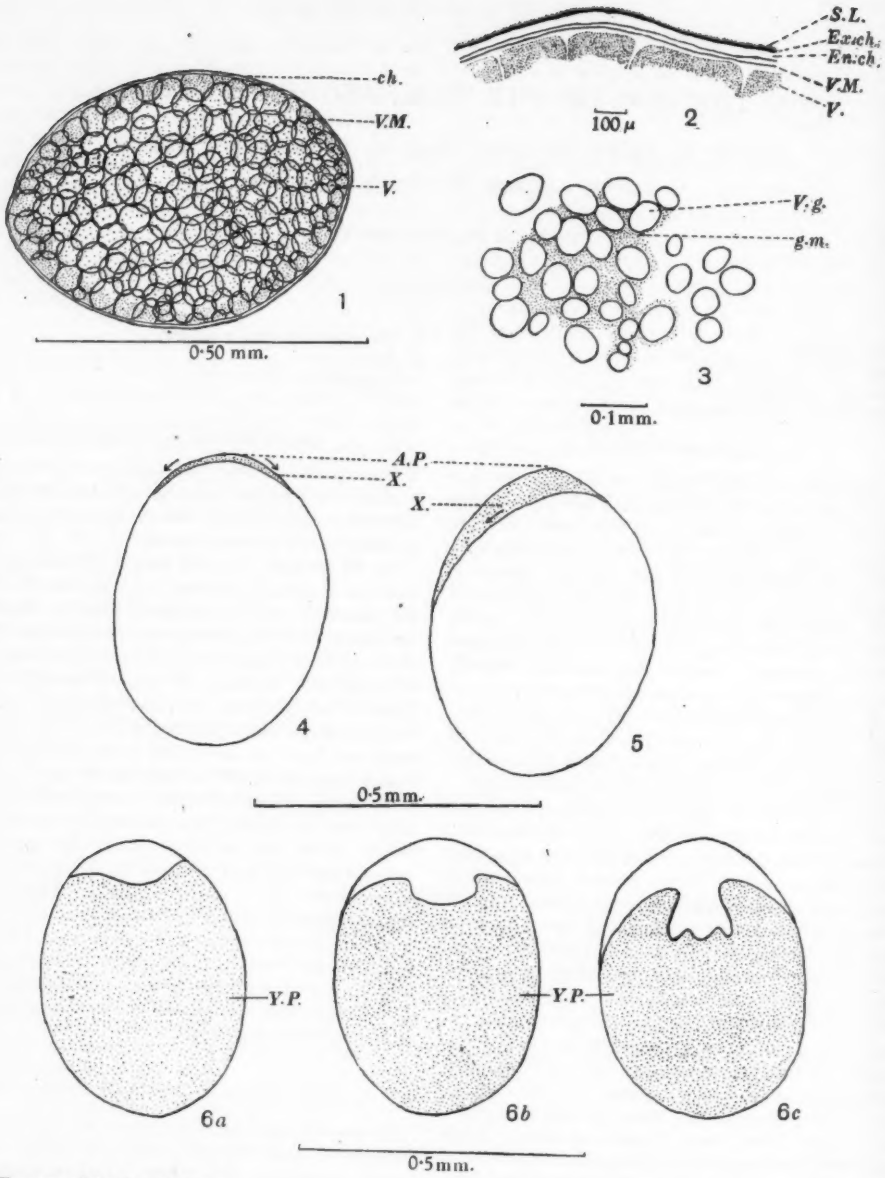


Fig. 1. The egg of *Ixodes ricinus* L.

Fig. 2. Transverse section of egg membranes of *Ixodes ricinus* L.

Fig. 3. Globular contents of egg of *Ixodes ricinus* immediately after oviposition.

Fig. 4. Initial visible differentiation of egg (dorsal view).

Fig. 5. Initial visible differentiation of egg (lateral view).

Fig. 6. Stages in the development of the yolk 'plate' (see text) from the dorsal aspect.

Key to Lettering: *A.P.* anterior pole of egg; *a.b.* air bubble; *cap.* capitulum; *ch.* chorion; *che.* chelicerae, overlying hypostome; *E.C.* embryonic cuticle; *E.S.* excretory sac; *Ex.* excretory globules; *En.ch.* endochorion; *Ex.ch.* exochorion; *g.m.* granular material; *L1*, *L2*, *L3*, legs 1, 2 and 3; *P.* palp; *S.L.* secretory layer from Gené's organ; *V.* vitellus; *V.g.* globules in egg contents; *V.M.* vitelline membrane; *X.* initial area of visible differentiation; *Y.P.* yolk 'plate', as seen in dorsal view.

crevices, or in the centre of the egg mass, results in a certain amount of flattening.

All measurements were made on fresh eggs under a binocular microscope. The length was measured as a straight line joining the two poles of the egg, and the width as the greatest diameter of the egg midway between the two poles. In *I. ricinus* the eggs were measured at three stages of development, comparable samples being taken in each case. The stages were measured as follows:

(1) Freshly laid eggs. From the time of oviposition to 24 hr. later.

(2) Partially developed eggs. At about the 33rd–35th day after oviposition.

(3) Fully developed eggs. Usually from batches from which some eggs had already hatched.

Bischoff (1927) is of the opinion that the increase is due to the intake of food material by the embryo, but the writer believes that it is due to the absorption of water from the surrounding medium, the significance of which will be dealt with later, in connexion with the cuticular membranes. It has been shown by McLeod (1935), Milne (1944) and others that the maintenance of high humidity (over 80 %) in the microclimate is essential for the development of the immature stages of this tick, including the egg phase. It is well known that eggs of ticks soon shrivel up, with the collapse of the egg wall, under dry conditions. It is of interest to note that the eggs of the majority of the insects, in which this size-increase phenomenon has been observed, develop under conditions of high humidity, in many cases approaching saturation point.

Table 1. *Length of eggs of* Ixodes ricinus (*in mm.*)

	Frequency in mm.							Total no. of samples	Arithmetic mean	Standard deviation	Standard error of mean
	0·52	0·54	0·56	0·58	0·60	0·62	0·64				
(1) Freshly laid eggs	3	12	37	64	49	14	1	180	0·575	0·20	0·015
(2) Eggs on 33–35 day of development	—	17	19	32	78	31	3	180	0·586	0·17	0·013
(3) Fully developed eggs (actual)	—	—	1	23	49	22	5	100	0·597	0·017	0·0017
(4) Fully developed eggs based on 180 samples	—	—	1·8	41·4	88·2	39·6	9	180 (estimated)	—	—	—

Table 2. *Width of eggs of* Ixodes ricinus (*in mm.*)

	Frequency in mm.				Total no. of samples	Arithmetic mean	Standard deviation	Standard error of mean
	0·41	0·43	0·45	0·47				
(1) Freshly laid eggs	14	39	47	—	100	0·440	0·04	0·004
(2) Eggs on 33–35 day of development	5	33	39	3	100	0·446	0·02	0·002
(3) Fully developed egg	2	27	66	5	100	0·449	0·01	0·001

The frequency distribution of the samples with regard to the length and width are presented in Tables 1 and 2 under 0·2 mm. magnitude classes. There is an apparent departure from normality due to the grouping effect, and the means are calculated as for a normal population distribution. From these data it is seen that the egg increases in size with development. The length increases from 0·575 to 0·597 mm., a total mean increase of 3·8 %; but there is no indication if this differs before and after the 33rd–35th day period. Correspondingly there is an increase in width from 0·440 to 0·449 mm., a total mean increase of 2·04 %, this being twice as marked before the 33rd–35th day as after.

The swelling of eggs, bounded by a firm pellicle such as the chorion, is a relatively rare occurrence, although it has been recorded by some authors in particular in the Orthoptera, Dermaptera, Rhynchota, Hymenoptera, Coleoptera and Diptera.

In Tables 3 and 4, on the basis previously indicated, the length and breadth of the eggs of *I. ricinus*, *I. hexagonus* and *I. canisuga* are given for comparative purposes. All dimensions apply to eggs oviposited up to 24 hr.

Whilst it has been shown (Tables 1 and 2) that there is a variation in size between eggs at various stages of development, it is equally apparent from Tables 3 and 4 that there is a variation in egg size both between the three species of the genus and between those of individuals of the same species directly on oviposition. In one case, for example, it was noted that from an exceptionally laterally distended and dorso-ventrally depressed female of *I. ricinus* the mean length of the eggs directly on oviposition was 0·70 mm., with a range from 0·65 to 0·75 mm., and the mean breadth of 0·49 mm. with a range from 0·47 to 0·54 mm. As development proceeded, the examination of the eggs showed the

increase to be of the same order as that indicated in Tables 1 and 2. Despite the existence of this variation in the eggs, there is no evidence from the information collected that the egg size varies according to the time at which they are deposited during the oviposition period, i.e. between those deposited at the commencement and the end of the ovipositing season.

Immediately after egg deposition the individual egg of *I. ricinus* weighs 0·00006 g.

Table 3. *Length of eggs of* Ixodes ricinus, I. canisuga *and* I. hexagonus (*in mm.*)

Species	Frequency in mm. 0·48	0·50	0·52	0·54	0·56	0·58	0·60	0·62	0·64	No. of samples	Arithmetic mean
I. ricinus	—	—	3	12	37	64	49	14	1	180	0·575
I. canisuga	12	32	35	189	43	12	0	2	—	325	0·540
I. hexagonus	3	2	79	140	199	54	3	1	—	481	0·558

Table 4. *Width of eggs of* Ixodes ricinus, I. canisuga *and* I. hexagonus (*in mm.*)

Species	Frequency in mm. 0·39	0·41	0·43	0·45	0·47	0·49	0·51	No. of samples	Arithmetic mean
I. ricinus	—	14	39	47	—	—	—	100	0·44
I. canisuga	5	49	326	60	2	—	—	442	0·43
I. hexagonus	2	0	2	163	24	13	1	224	0·44

4. THE MEMBRANES OF THE EGG

When the egg is laid direct from the oviduct it is enclosed by two envelopes, the chorion and the vitelline membrane. The egg shell, or chorion, consists of two layers, the exo- and endochorion (Fig. 2), which differ in structure and properties. The exochorion is applied like a thin varnish over the egg surface and stains less deeply with acid fuchsin than does the endochorion. It is less compact than the latter and in some examples is only feebly developed. The endochorion, lying beneath the exochorion, is a relatively thick, opaque, elastic layer, which gives the egg its shape and partly its consistency and it may be regarded as the 'egg shell'. It is of nearly uniform structure and thickness over the whole egg. In some cases it has been noted that the exochorion peels off with the swelling of the eggs, and there is some evidence that between the exo- and endochorion there is an oily or lipoid layer. At least it can be said that the two membranes are quite unconnected and that the exochorion, when removed, leaves the endochorion with a shiny, smooth, glistening surface. Within the chorion one finds the vitelline membrane, which is clearly visible when the egg is mounted. Initially the vitelline membrane appears as a structureless basement membrane which is closely adherent to the cortical layer of protoplasm beneath it. During the early stages the vitelline membrane came away with the chorion when the latter was dissected from the egg. With the development of the egg it is commonly seen as a thickened envelope showing a distinctive laminated appearance (Fig. 11).

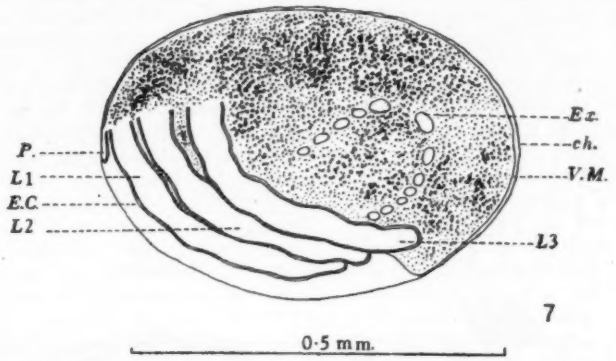
Each egg is surrounded by a secretion immediately on oviposition, this being produced by Gené's organ (Nuttall, 1911). It has been observed previously that, following damage to this organ, oviposition ceases for a while and, when continued, the eggs are laid directly from the oviduct. Such eggs are not covered with a secretion, with the result that they soon shrivel and dry up. Similar observations were apparent when Gené's organ was not pricked but simply stimulated with a fine glass rod. Gené (quoted by Nuttall, 1911) supposed that in addition to covering the egg, this secretion has the further function of hardening the 'chitinous' envelope of the egg, and that the organ may convey spermatozoa in some way from the female uterus to the egg as she is laying. This secretion forms an additional layer to the egg membranes, although it is derived from accessory glands. Probably this secretion also acts as a cement to hold the eggs together in a cluster. Single eggs, or small clusters, may be removed from the parent cluster by means of a needle, and if care be taken, the exochorion need not be torn or visibly damaged. Further, if after being so separated, eggs are examined, no mark indicating where the eggs have been in contact can be seen.

Individual eggs, removed from the parent cluster with care, show the surrounding gel-like layer, which in a saturated atmosphere appears to attract moisture, and globules of water may be found adhering to it. When immersed in solutions of NaCl (0·001–0·06 %) the secretory covering swells up to a width considerably greater than that of the chorion itself. It appears, however, to be of a waxy nature because, if egg clusters are treated with ether, they rapidly disintegrate. The removal of a portion of the secretory layer results in an increased loss of moisture and, where such an area is extensive, the egg becomes desiccated.

5. DEVELOPMENT AND HATCHING

From a survey of the literature it would appear that the only controlled experiments on the development of the egg of *I. ricinus* are those by Olenev (quoted by McLeod, 1935). He observed that whereas under natural conditions eggs took 8 weeks to hatch, under artificial conditions at 25–30° C. they hatched in 3 weeks. Death ensued when the relative humidity fell below 80 %. McLeod (1935) has shown that the lower limiting humidity for egg development corresponds with the later stages, being in the neighbourhood of 80 % saturation. Under the latter conditions it has been shown (Arthur, 1945) that the head of the emerging larva is flexed ventrally and is not visible from the dorsal aspect. This is not so at or near maximum saturation limits according to Campbell (1946); the latter also notes that when eggs are subjected to temperatures above 25° C., a typical abnormality encountered in those individuals which survived and hatched is a persistence of the embryonic dorso-ventral curvature of the whole length of the body. Again, the mouthparts are not visible when viewed dorsally.

The following observations on the process of development of the tick egg to the hatching stage were undertaken on living eggs which were maintained in the laboratory at temperatures ranging from 16 to 23° C. in a saturated atmosphere. The preoviposition period varies with the season and with the individual, but usually lasts 15–22 days (McLeod, 1935). Detailed observations on the early development phases of the living eggs are not possible owing to the density of the internal contents. A contraction of the cytoplasm and yolk from the anterior pole is the first external evidence of development; this when seen dorsally is in the form of a crescent with backwardly directed horns (Fig. 4). Laterally the direction of development is ventro-posteriorly and is most emphasized in this plane (Fig. 5). The process of external change is a gradual one and follows the path indicated by the arrows in the figure. Dorsally



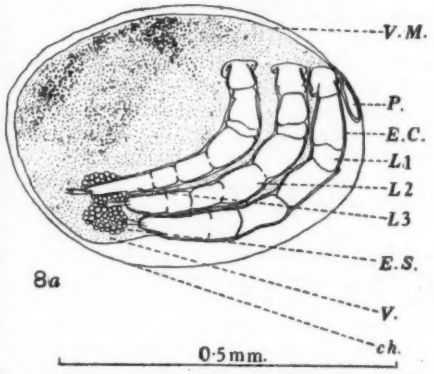


Fig. 7. Lateral view of developing tick in egg between 30 and 39 days at conditions of experiment (see text).
Fig. 8*a*. Lateral view of developing tick between 39 and 51 days at conditions of experiment (see text).

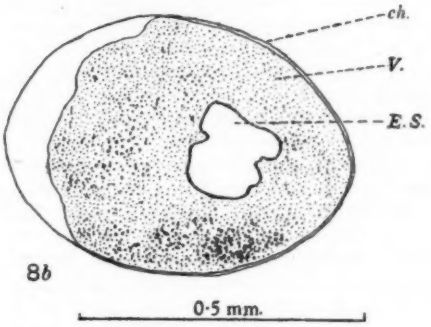


Fig. 8*b*. Dorsal view of egg, showing excreta visible through the integument (37–43 days). (Key to lettering on p. 54.)

there appears to be a distinct 'plate' (Fig. 6*a–c*) which, with increased differentiation, shows a gently concave anterior border, this concavity becoming more accentuated as development proceeds. This plate corresponds to the dorsal distribution of the vitellus in the egg. The less dense layer immediately anterior to the yolk 'plate' corresponds to the future cephalothorax and appears to be the centre of differentiation. When once this has commenced its activity, it provides the focus from which all subsequent processes of development spread, although in an undeveloped egg it displays no visible difference from any other part. A similar centre to this is present in insects (Wigglesworth, 1939), which is said to act 'not by giving out a diffusible substance, but by initiating a contraction wave in the yolk system, spreading forwards and backwards and forcing the blastoderm cells, hitherto distributed uniformly to fill the space that is left'.

The legs in their first stage are unsegmented and enclosed within a membrane, the first or 'embryonic' cuticle which surrounds each limb in an unsegmented sac (Fig. 7). Owing to the shortness of the sacs the legs are sinuate. When seen from the ventral view the appendages are closely apposed to one another and to the body generally. The first pair of legs lie close against each other on either side of the mid-ventral line. The tips of the second pair are almost level with those of the first pair and the third pair of legs extend further to the posterior.

Thirty to thirty-five days after oviposition a number of globules are visible in the internal brown mass. From a lateral view these globules are arranged in the form of a horseshoe (Fig. 7), the

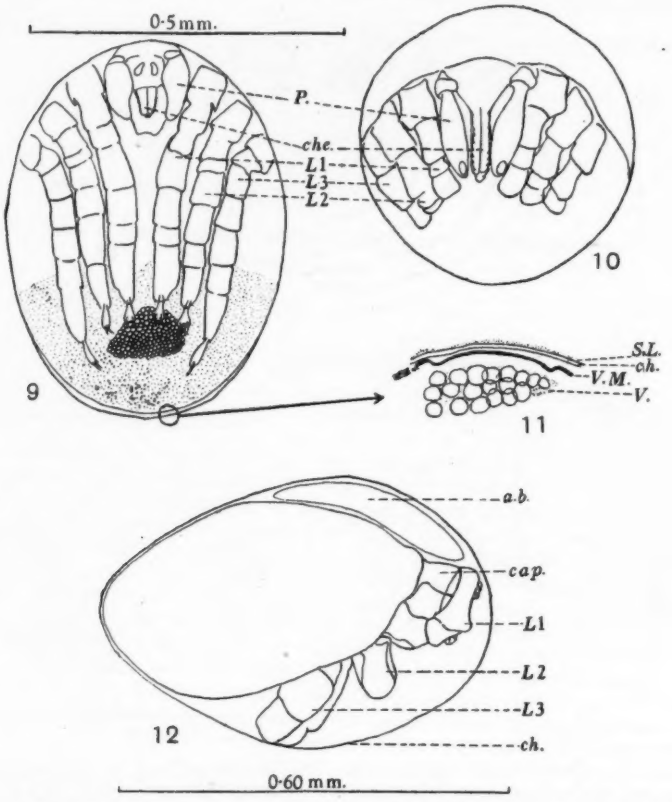


Fig. 9. Ventral view of developing tick in egg to show position of mouthparts.

Fig. 10. The same embryo as seen at the anterior pole, showing the capitulum and palps orientated at 90° to the longitudinal axis of the egg.

Fig. 11. Section through the egg membranes at the hatching stage showing thickened vitelline membrane (compare with Fig. 2).

Fig. 12. Larval tick preparatory to emergence. (Key to lettering on p. 54.)

ventral limb of which leads to the neighbourhood of the apex of legs 1 and 2. By the 37th–43rd day, when viewed under reflected light, the eggs show an extensive white area (Figs. 8*a*, *b*), in the vicinity of the tips of the legs. This white mass consists of globules of uric acid, enclosed in a sac formed at the junction of the gut and the Malpighian tubules. This sac, when distended with this excreta, is readily visible through the transparent egg shell. The aforementioned globules, arranged in a horseshoe pattern (Fig. 7), are the initial waste products on the way to the sac. The legs now show distinct evidence of segmentation, and at this stage the capitulum and palps are clearly visible, lying at an angle of about 90° to the longitudinal axis of the tick body (Figs. 9, 10).

During the 48 hr. before emergence, the body of the tick comes to fill the egg more completely, and is bathed in the amniotic fluid. Occasionally, rhythmic movements of the head are seen, and it is suggested that at this period the larva swallows the amniotic fluid in much the same way as insects do. The gut is filled with the remains of the vitellus in the form of small globules and granular material. When the amniotic fluid mixes with the gut contents the small globules unite to form relatively large droplets. All the free fluid in the egg is consumed and the larva increases considerably in size before hatching. Within 24 hr. of hatching the outward form of the egg changes, becoming longer and thinner. Preparatory to emergence the mouthparts are directed forwards and are but slightly depressed ventrally from the longitudinal axis. This depression is also a common feature in mature larvae. The legs, having now ruptured the 'embryonic' cuticle, are no longer apposed to the body (Fig. 12).

Air enters the egg, presumably by diffusion through the shell, and occasionally some air may be swallowed by the larva. This, however, is not a constant process and does not seem to play an essential part in the mechanism of hatching. According to Sikes & Wigglesworth (1931) the swallowing of air may (i) distend the larva while still inside the egg, and so assist the movement of body fluids; (ii) in those cases in which the larva escapes from the egg while still enclosed in the embryonic cuticle, it may swallow air a second time, blowing itself up until the cuticle is forced to split; and (iii) the young larva may swallow air until its skin has stretched and attained a size much greater than the egg from which it came. The two latter considerations do not apply to the tick *I. ricinus*, and the inconstancy of the swallowing of air does not lend much support for (i).

There is no evidence in the tick that there is an increased pressure inside the egg consequent on embryonic development. The egg is turgid, and though it can be deformed by external pressure at this advanced stage without any effect on the larva, the shell returns to its normal shape by its elasticity.

In the pre-eclosion phase the larva extends the posterior part of the body against the chorion, and the initial splitting takes place at this posterior point from whence it passes forwards. The initial force employed to break open the egg appears to be muscular, the posterior border of the abdomen lies in close apposition to the chorion and any abdominal muscular movements would tend to increase the force against the shell at this point (Fig. 12). There is reason to believe that the rupture of the egg shell along the longitudinal axis forms a distinct line of weakness, as similar cleavages have been obtained by the treatment of newly deposited eggs with dilute and concentrated acids.

No air appears to be swallowed after hatching. The posterior end of the body invariably emerges first, and the anterior portion may be retained for some hours within the egg shell. Ultimately, by slow movements of the legs, the entire body comes out and the larva crawls a short distance from the egg shell. At the experimental conditions referred to in the present paper, the ticks remained quiescent for 5–10 days. During eclosion the excretory sac is charged with waste matter, and the newly emerged larva continues to discharge the contents over a period of several days until the sac is completely emptied. Elimination of excretory products usually begins at the time of hatching, and frequently a quantity of waste material remains in the egg shell. After emergence the gut undergoes a well-defined series of changes, simultaneously with the process of excretion and hardening of the chitinous integument. In the newly emerged larva the gut frequently appears as a simple sac charged with yolk (the 'plate' observed earlier), but within a short period of time the yolk is mostly digested and the typical lobed appearance is assumed. Essentially comparable developmental processes are apparent in *I. hexagonus* and *I. canisuga*, and the process of hatching is similar in all three.

5. SUMMARY

The egg of *Ixodes ricinus* L. is described, and the increase in size during development is noted. It is suggested that the increase is due to the absorption of water. The egg wall consists of exo- and endochorion with a thin vitelline membrane, which becomes thicker as development proceeds and assumes a laminated appearance. The secretion layer of Gené's organ is described, and appears to function as a cement for holding the eggs together in the cluster. Chemical tests show it to be of a waxy nature.

It is deduced from experimental evidence that the egg shell is not absolutely rigid, and the weight of evidence favours the view that the shape of the egg shell is maintained by the internal pressure of the egg contents, and the elasticity of the shell.

The development processes as seen in the living egg are described. Hatching from the egg shell is brought about by a muscular force acting on a limited area at the posterior pole of the egg.

The writer is indebted to Mr Robert David, of Longlands Farm, Pyle, Dr A. D. Lees, of the Agricultural Research Council, and Mr Simeon, of the Glamorgan War Agricultural Executive Committee, for supplies of material; to Mr T. W. Tyssul Jones, M.Sc., for his assistance in checking the measurements of the egg dimensions, and to Mr L. Cowley, M.Sc., Assistant Curator (Department of Zoology), National Museum of Wales, and Dr P. Tate, Molteno Institute of Parasitology, Cambridge, for reading the original script and for making many useful suggestions and modifications.

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THE DISTRIBUTION AND DYNAMICS OF KED POPULATIONS, *MELOPHAGUS OVINUS* LINN.

By JOHN MACLEOD, *Cooper Technical Bureau, Berkhamsted*

(With 4 Figures in the Text)

The life history of the sheep ked, sometimes called the sheep tick, has been studied by a number of workers, notably Swingle (1913) in America and Hill (1918) and Graham & Taylor (1941) in Australia. A preliminary note on recent work in Britain has been published by Evans (1946). The present record is intended to supplement these exact studies, and confines itself to observations on the ked populations as a whole, and on the changes in density and distribution of such populations. It is hoped later to relate the results to the types of habitat and to the climatic and other changes in these habitats with changing season.

The observations were made at the Cooper Field Research Station, west Hertfordshire, during the period 1939–42.

The study of natural populations of keds is extremely time-consuming, since the whole fleece of each sheep must be gone over thoroughly, and since reasonably large groups of sheep are required on account of the wide scatter of individual results. The difficulty is increased by the matting of the fleece where puparia are attached, and by the necessity for checking whether pupa cases are unhatched or empty; this often involves testing by feel between finger and thumb, and where it is required to leave the population as undisturbed as possible, this pressure test has to be done very carefully, to avoid either crushing or loosening the attachment of the puparium.

The considerable increase in the time required compelled me to abandon the sexing of the adults, so the following records refer only to total adults and puparia.

Opportunities of supplementing the results of 'live' counts, by counts of killed populations occurred as incidental to experimental work on control by dipping; details are given in the text.

RELIABILITY OF 'LIVE' COUNTS

If a preliminary assessment of the adult and pupal population is made on a group of sheep, and the sheep are then dipped in a quick-acting insecticide, the dead keds may be counted on the following day and will give a check on the accuracy of the preliminary count of adults. If these dead keds are removed, and periodic counts made during the following 24 days, all observed keds, living or dead, being removed at each count, the total of such periodic counts will represent the pre-dipping pupal count, less those puparia which have been sterilized by the dipping, and, of course, less any normal pupal mortality. Neither check, of course, takes account of any adults or puparia washed out of the fleece.

The method was used with a coal-tar creosote dip, but gave unsatisfactory results, from two causes. The possibility of a partial sterilizing effect on puparia is difficult to verify and its extent difficult to measure. Secondly, with this type of dip a considerable number of keds and particularly of puparia are washed out of the fleece during the actual dipping. Subsequent to the dipping, also, many dead keds appear to be lost from the drying fleece. These effects are possibly related to the soap emulsifier used in the dip. The figures were:

20 sheep: keds observed 1551; recovered after dipping 1234.

10 sheep: puparia observed 625; recovered by periodic counts 688.

More satisfactory results were obtained with a straight derris resin dip, with proteo-borax emulsifier. Derris kills adult keds, and remains effective in the fleece for over 24 days. It has no sterilizing effect on puparia, but kills the adults as they emerge and before their abdomens are fully expanded. Such newly emerged dead adults are thus readily recognizable.

Ten sheep gave counts of 690 adults and 636 puparia. They were dipped the same day. The sheep were examined and dead keds removed on the 3rd, 11th, 20th and 26th days after dipping. The numbers of keds recovered at each count were:

3 days	11 days	20 days	26 days	Total	Total pre-dipping count
784	434	448	230	1896	1326

The emergences from the 3rd day onwards total 1112. Assuming emergences to continue for 24 days, this gives a daily emergence rate of 53. Thus, of the 784 recovered on the first post-dipping count, 160 probably emerged after dipping, leaving 624 to

represent the 690 observed before dipping. The discrepancy is not excessive, and is probably due largely to mechanical loss at dipping. The pre-dipping survey of the adult population appears from the figures to have been comprehensive, and the 'live-count' method would thus seem reasonably reliable for estimating adults. The recovery figures after the 3rd day give the following emergence rates:

Days	Total	Rate per day
4–11	434	54
12–20	448	50
21–24	230	58

The consistence of the emergence rates suggests that the counts were reliable, i.e. that practically all the dead keds present in the fleece were being recovered at the periodic counts. The total of 1112, with 160 added for the first 3 days, makes a total of 1272 as against the pre-dipping assessment of 636 puparia.

It is necessary at this stage to carry the analysis a step further and examine the reliability of the reliability check—specifically, the possibility of dead keds being lost to the count by dropping out of the fleece between periodic counts. The average longevity of males and females is about 80 and 100 days respectively (Graham & Taylor, 1941). If a general figure of 90 days is taken, the death-rate in a stable population is $\frac{1}{90}$ per day, or 1·1 %. The ratio of dead to live keds in a normal fleece, divided by 0·011, is, therefore, a theoretical measure of the number of days dead keds remain in the wool.

In April 1942 two normal, i.e. undipped, flocks of ten Suffolk crosses and seven Dorset Horn sheep, which have a closer, more compact fleece, were examined and the proportion of dead to living keds estimated. The results are given in Table 1.

There is a very wide dispersion of the ratios of dead keds in the individual sheep; in a distribution of this type only seven in ten of the observations would be expected to fall in the range zero to double the mean. The mean ratios are 12 and 4·5, the ratio of the totals 7·5 and 5. If the lower ratio for the open-woolled sheep is taken, the figure means that dead keds remained about 7 days (7·5/1·1) in the fleece. With the fairly open-woolled sheep used, there is thus a reasonable expectation that any one dead ked would be retained for a week, but an appreciable proportion of the total for the groups of sheep would be lost. The theoretical proportions lost for each interval between counts may readily be calculated from Table 1, but, with such limited data, the precision would be quite unreal.

To return to the 'recovery' check on the pre-dipping pupal count, it follows from the above that the total of 1272 is short of the actual emergences by an unknown but appreciable amount. Thus, the pre-dipping assessment of 636 puparia is probably no more than 50 % accurate. The results of the 'live-count' method for puparia are not, therefore, comparable in accuracy to those for adults, and the observations on pupal populations reported here should be regarded more as an indication of the probable relative distribution, and of the trend of changes in distribution, rather than as values comparable with the corresponding adult values.

Table 1. *Ratio dead to live keds in normal fleece*

Suffolk Crosses			Dorset Horn		
Live	Dead	Ratio (%)	Live	Dead	Ratio (%)
23	3	13	26	2	7·5
23	7	30·5	32	0	0
26	3	11·5	43	0	0
32	10	31·5	45	0	0
61	6	10	53	5	9·5
86	7	8	63	3	5
92	3	3·5	68	6	9
102	4	4	—	—	—
110	5	4·5	—	—	—
117	2	1·5	—	—	—
Mean 67·2	5	12 ± 11	47·1	2·3	4·5 ± 4·5
Ratio totals (%)	—	7·5	—	—	4·9

RELIABILITY OF MEANS

The frequency curve of the adult population is slightly biased by a positive tail of high values, but not sufficiently so to justify logarithmic transformation, which with most of the groups produces a still more pronounced positive bias. The general shape of the curve is low and flat, the relative shape being fairly constant for populations at different density levels. Table 2 gives the coefficients of variation (the standard deviation as a percentage of the mean) of nine samples with means ranging from under 10 to over 200. The coefficients remain remarkably uniform throughout this range.

The low shape of the distribution curve results in the means of groups having a rather wide sampling error; the standard errors for the groups in Table 2 are, with two exceptions, between one-eighth and one-quarter of the mean values.

Table 2. *Dispersion of individual counts about the mean (adult keds)*

Mean	No. of sheep	Coeff. var.	S.E.
6·67	6	45	1·23
8·56	9	80	2·29
11·20	10	54	1·93
45·48	21	61	6·05
69	15	41	7·28
77·55	20	31	5·35
82·86	7	37	11·5
142·70	17	59	20·5
219·10	18	53	27·2

DISTRIBUTION OF POPULATIONS OVER THE BODY

The body areas referred to in this section are:

Back. From the back of the head to the tail, extending about 4 in. on either side of the spine, and about 4 in. broad on the neck.

Chest and Forequarters. The throat and chest to the brisket, and the forelegs, to the margin of the dorsal strip.

Hindquarters. The breech to the perinaeum, and the hindlegs.

Ribs. From the fore- to hindleg, extending from the dorsal strip to the margin of the belly wool.

Abdomen. From the brisket to the udder or scrotum.

The distribution on ten half-bred (Cheviot-Leicester cross) adults and seven half-bred lambs in the spring of 1939, and on a mixed flock of nine cross-bred adults in the summer of the same year, is illustrated in Fig. 1. The figure shows the general correspondence of the adult and pupal distributions. In the spring the infestation was heaviest on the chest and rib wool in the adult sheep, while the lambs, with a less heavy fleece, tend to have the heaviest concentration in the chest area.

All three flocks were running together during the spring and summer, and the markedly lower infestation in June, before shearing, indicates a reduction rather than a continued low infestation. Evidence of such a seasonal reduction is given later. The apparently greater proportion of puparia to adults in summer than in spring is probably due to the more accurate assessment of the pupal population with low infestations.

In early May 1941, as incidental to a dipping experiment, further estimates were made of the adult population, this time by the 'recovery method', i.e. the counting of dead keds 2 days after dipping. The sheep were two flocks of seventeen Kent cross adults and eighteen Suffolk cross and Hampshire cross adults, bought in the open market shortly before. The second flock consisted of two groups of twelve and six, with different histories. The distribution of the adult population on the three groups is illustrated in Fig. 2. The relative distribution is fairly uniform for the three except that the Kent crosses have a higher concentration in the chest and forequarters wool; the distribution as a whole differs from that shown in Fig. 1 (March) in the greater proportion of keds in the hindquarter than the forequarter region, and in the fairly heavy infestation of the dorsal wool.

The dip used was a coal-tar creosote plus derris-root emulsion, the exact effect of which on the puparia is unknown. An estimate of the puparia present at dipping, based on subsequent recovery of adults at periodic examinations, was made on the Kent cross flock; it serves for comparison of the relative distributions, but the values cannot be regarded as indicating the adult-puparia ratio. A total of 1258 pupae was estimated for fifteen of the sheep. The percentage distributions of adults and puparia are:

	Chest and fore-quarters	Back	Ribs	Abdomen	Hind-quarters
Adults	24	10	39·5	8·25	18·25
Puparia	23·5	6·25	40	11·25	19

The only appreciable differences are in the dorsal and ventral areas, the puparia being relatively more abundant in the abdomen area.

Since the mean infestations of the other areas are clearly significantly higher than those for the back and abdomen, i.e. do not belong to the same statistical population, these two can be compared directly for significance of the difference between them, i.e. without regarding them as two out of five samples. Comparing the two groups as a whole, the increase of puparia in the abdomen is between the 1 and 2 % points. The adult preponderance in the back area is not significant, P being nearly 50 %, but if the adult counts are compared as a series of pairs (back against abdomen on each sheep), the probability of randomness is reduced to between 10 and 20 %. Since the differences for adults and puparia are in opposite directions, the differential distribution is clearly significant. Examination of the totals for each area as a fourfold table gives a probability of less than 1 % that the adults and puparia are randomly distributed over the two areas. In other words, there is a significant tendency for the lower surface to be used as a breeding area to a greater extent, relative to the adult distribution, than the upper surface.

SEASONAL CHANGES IN DISTRIBUTION OVER THE BODY

A series of counts, at approximately monthly intervals, was made on a mixed flock of ten Suffolk cross and Hampshire cross sheep from May 1941 to June 1942. The flock was shorn after the June count, and in August a flock of six Suffolk cross lambs, brought to the farm in July, was run along with them. Parallel counts were made on the lambs from August until March, to study the changes in unshorn sheep and the course of reinfestation of the shorn adults. For this experiment the body areas were subdivided as shown in Fig. 4.

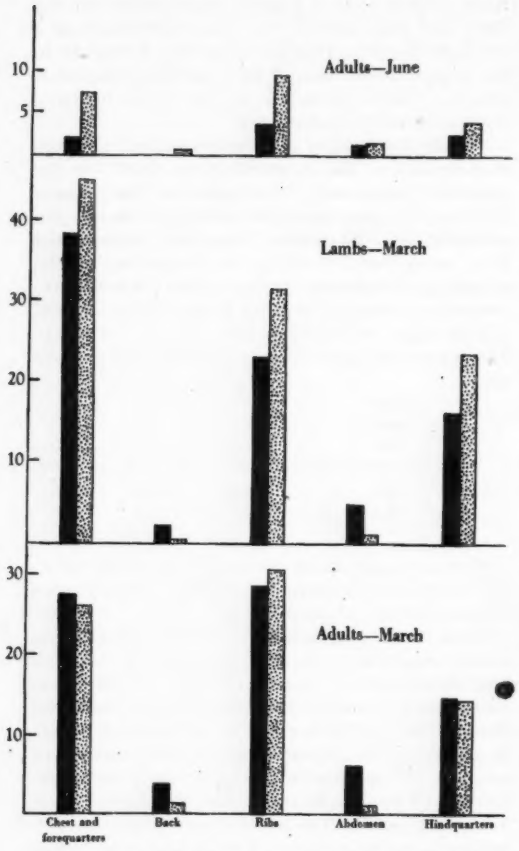

Fig. 1. The distribution over the body of adult keds (black columns) and puparia (stippled). March, means of ten half-bred adult and seven half-bred lambs. June, mean of nine mixed cross adults. Estimates by 'live-count' methods.

By April the infestations of the six hoggs were so heavy that they were discontinued from the experiment and dipped. After the October count six of the ten adults were dipped, as a routine procedure. The adult sheep counts for December, January and February are on four sheep only. By March, however, the infestation of the dipped sheep was not significantly lower than that of the undipped, and the March and later counts are on ten sheep. Fig. 3, showing the mean total count of adults and puparia on the sheep and lambs, illustrates three points of interest. There is a distinct drop in infestation density in the adult sheep, between May and June in both years. The shearing, usually in June or early July, truncates the population curve, but is not the sole cause of the summer depression; there is clearly a seasonal biological factor operating. The lamb counts show a decrease in summer, but since this continues on until December, while the adult population has begun to rise after October, it is not clear whether the lamb decrease is due to continued one-way migration, the adult fleece being too short to

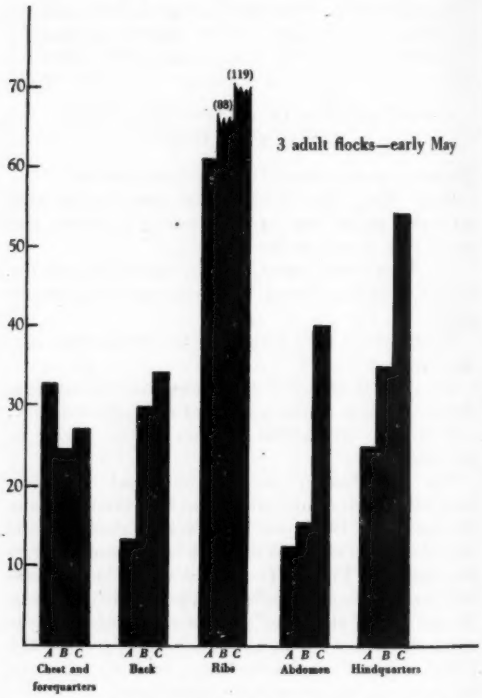

Fig. 2. Distribution (adults only) on: *A*, a flock of seventeen Kent cross sheep; *B* and *C*, twelve and six mixed Suffolk and Hampshire cross sheep. Estimates by recovery method.

support a flourishing population, or whether it is due to a constant controlling factor which would operate at this season even if the flock were isolated.

Both adults and lambs show an extraordinarily rapid increase in spring. The increase in this case was probably accelerated to some extent, since a number of newly acquired sheep were added to the farm flocks during the early spring, and doubtless increased the infestation level of the original stock, but this could account for only a small part of the total increase.

The changes in distribution throughout the year, from one pre-shearing count to the next, are shown in Fig. 4. The principal points of interest are as follows:

I. *Pre-shearing distribution.* Limited dorsal surface infestation, consisting entirely of puparia,

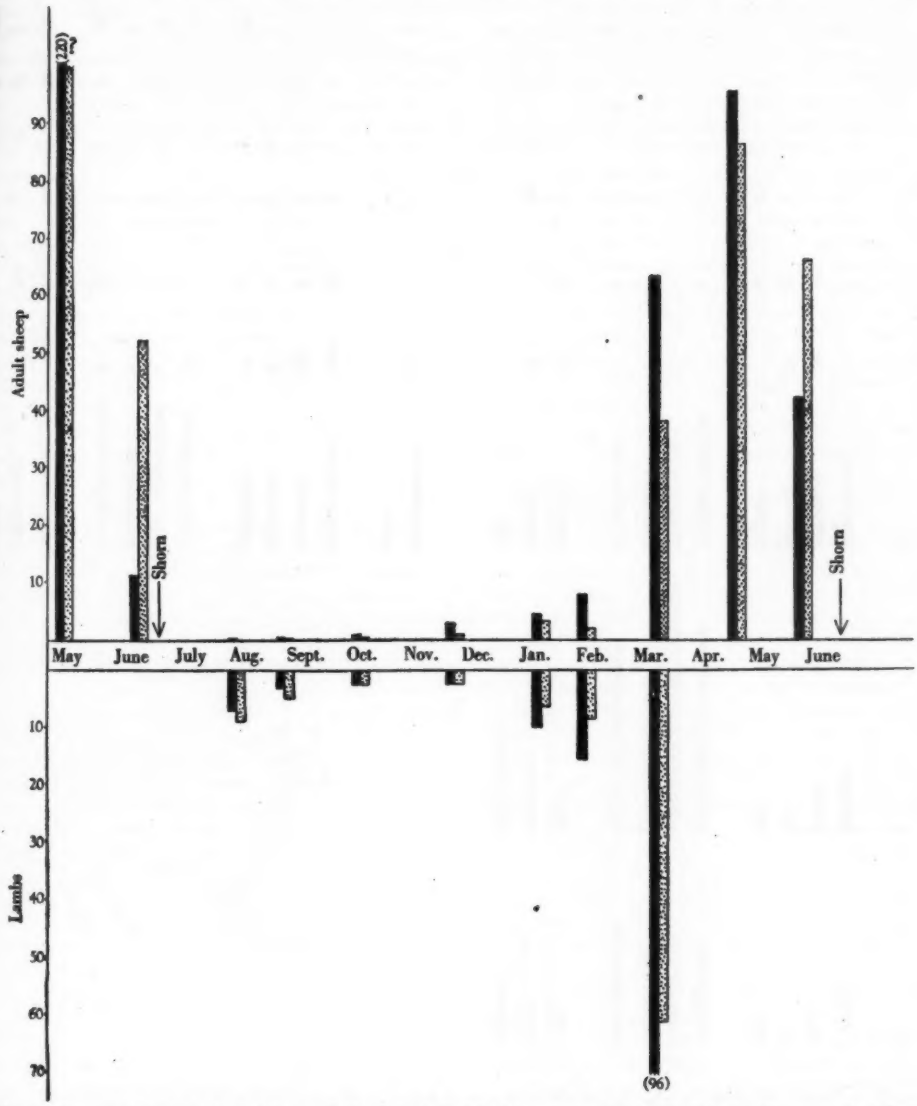

Fig. 3. Seasonal changes in total population of adult (black) and pupal (stippled) keds. Upper part of figure, means for ten cross-bred sheep; lower, means for six cross lambs.

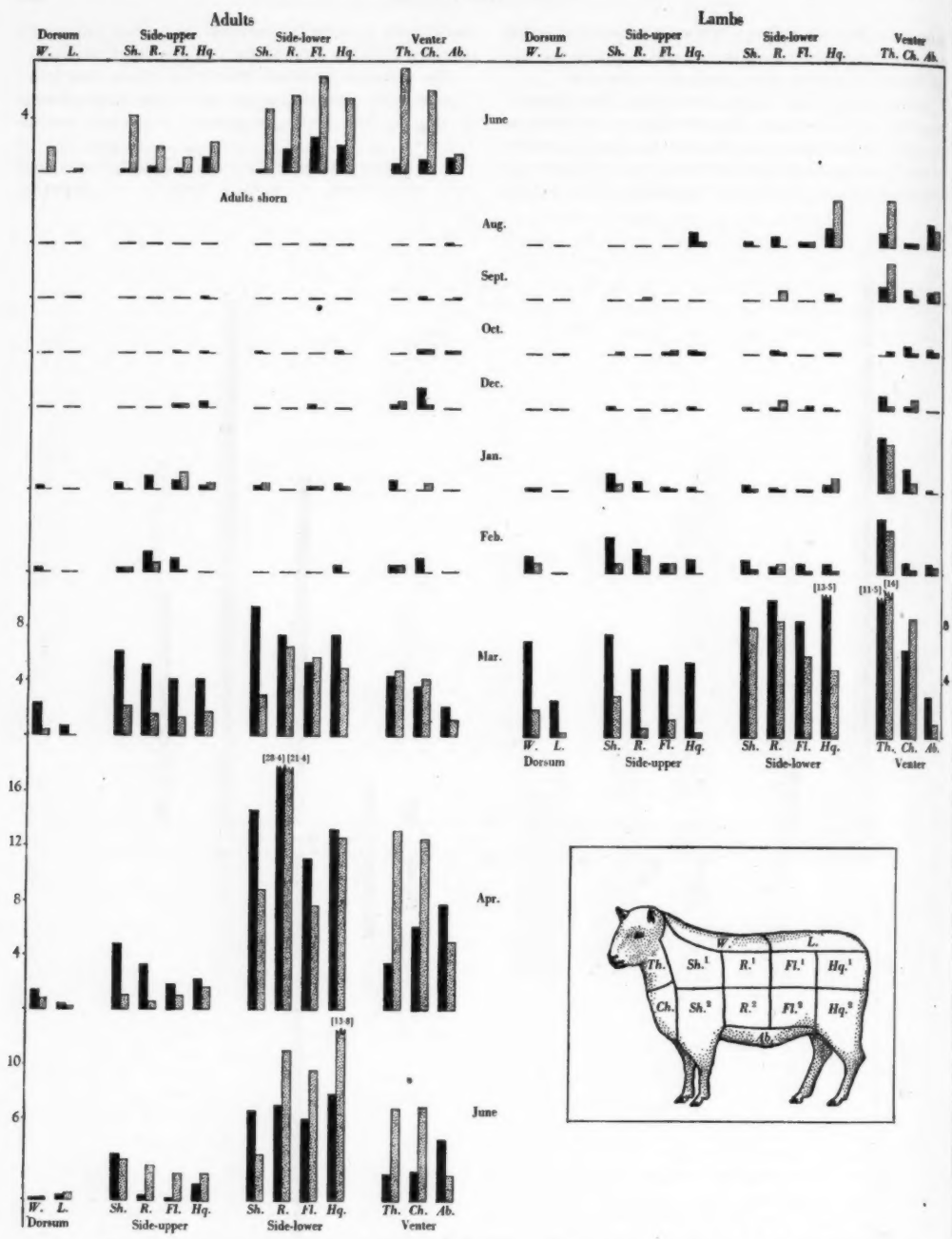

Fig. 4. Interpretation as for Fig. 3, but showing the changes in distribution over the body coincident with changes in total density. *Ab.* abdomen, *Ch.* chest, *Fl.* flank, *Hq.* hindquarters, *L.* loins, *R.* rib, *Sh.* shoulder, *Th.* throat, *W.* withers.

mostly in region of withers. (This is not repeated in 1942.) Upper curvature of side—few adults but a fair sprinkling of puparia, particularly in upper shoulder, which is continuous with withers region. (1942, adults in shoulder region.) Principal concentration in lower side wool.

II. *Summer and winter distribution on lambs.* Up to October, concentrated chiefly on throat, chest and abdomen, and on lower half of side wool. January—distributed fairly equally in both upper and lower side wool, and even spread on to dorsal wool, in withers region. February—a slight preponderance, even, in upper half of side wool, notably on shoulder and rib areas (corresponding to distribution on shorn sheep, whose fleece growth now well advanced), but highest infestation in throat wool.

III. *Autumn and winter reinfestation of shorn sheep.* Up to September, occasional evidence of transfer from lambs, but no successful establishment, i.e. no puparia. September and October—first puparia to appear are on ventral aspect. January—puparial infestation beginning to build up. Both adults and puparia mostly on upper curvature of side. Distribution continues into February, except for a slight relative increase in the throat and chest infestation.

IV. *Spring distribution, adults and hoggs.* Main shift in distribution is a concentration in the lower side wool and the chest, though the throat concentration of the hoggs remains high. Upper side wool infestation not proportionately increased, and the dorsal infestation still less so.

V. *Late spring peak distribution.* The progressive decrease in intensity—lower to upper side wool to dorsal wool—observed in early spring distribution, now much intensified. Absolute decrease in infestation of two upper aspects, in spite of a marked overall increase. Greatest concentration in the lower rib area. Little increase in the adult population of the throat and chest, but a considerable increase of puparia, suggesting these may be breeding areas. (The increase in infestation of the abdomen is more significant than the actual values suggest, because of the short and rather unsuitable nature of the belly wool.)

DISCUSSION

There is little information in the literature with which the findings recorded above can be compared. Graham & Taylor (1941) record that with experimentally infested sheep over 70 % of keds settled in the neck and shoulder regions. Of some 11,000 puparia noted, 45 % were attached on the chest area, the lower side wool being the next most favoured site. General observations indicated that with the onset of warm weather the earlier peak infestations dwindled rapidly, but the authors assume that the decline is associated with the development of a host resistance.

The work described in the present paper has brought to light two major facts which have a bearing on changes in ked populations as a whole:

I. There is definite evidence of changes in the distribution of keds at different times of the year. In winter and early spring the upper side wool is equally if not more favoured than the lower side wool, and an appreciable concentration occurs on the back. With the advancing year, the keds tend to forsake the upper surfaces, and the lower wool, including the abdomen—a site little favoured in winter—becomes the principal territory. The chest and throat are favoured throughout the year, but particularly in the winter and spring.

II. There is evidence of an early summer decline in the total ked populations. This was found in three years, and can be accepted as a definite seasonal effect. The shearing of a large proportion of the ordinary breeding flock results in the population on the lambs continuing to dwindle through unproductive one-way migration, since the shorn sheep cannot maintain an infestation and so there is no compensating back transfer. The total population of the flock thus probably reaches its nadir in late summer or autumn.

The early summer decrease of keds in woolled sheep may be due to three causes, singly or in combination: development of a host resistance, changes in the quality or quantity of skin secretions directly affecting survival or reproduction, and changes in the climatic conditions of the fleece. The fact that the decrease in total population is associated with changes in the distribution over the body suggests that the third explanation is the more likely, unless the food and/or fleece-quality factors show differential changes in the several areas. The apparent relative unsuitability in winter of the abdomen and to a less extent of the lower side wool may be related to the fact that these areas are frequently soaked with moisture, through the sheep lying on wet ground or other cause. The migration, with the approach of summer, from the upper aspects to the lower suggests a shift forced on the population by an external factor such as insolation, which in summer frequently raises the temperature in the fleece on the back and upper side curvature above the upper limit for pupal viability. The continuous occupation of the throat and chest area is consonant with both explanations.

Some information on the seasonal changes in fleece climate in different areas of the body surface has been collected during the period of the above observations, but since it is hoped to extend these, it is unprofitable to discuss the above considerations further in this report.

There is one economic implication of these studies

which deserves comment. Shearing, with British breeds of sheep, leaves practically no cover for keds or puparia. Shorn sheep are completely freed of their ked burdens. Re-establishment cannot, moreover, take place for some months after shearing; when it does it takes place from the lambs. The reverse relationship obtains in the spring; the lambs, at birth, cannot maintain an infestation, although they are often infested heavily, through continuous migration from the mother. In theory, therefore, if the section of the flock maintaining the population were rid of this parasite at either of these periods of reduced parasite stability, no reinfestation could occur, and the infestation should die out. In practice, the attack on the adult foci when the lambs are incapable of nursing a 'reservoir' population is not feasible, since even if it were practicable to dip ewes with lambs at foot, the lambs are not sufficiently of one age, and the oldest would have a considerable fleece growth before the latest were born. The converse is, however, quite practicable. During the summer, after the last 'stragglers' have been shorn and before the wool has regrown sufficiently to allow establishment, if all the unshorn, i.e. the lambs, were dipped in an effective insecticide, the ked population should be exterminated at a cost of the dipping of only about one-quarter of the stock. In practice two dippings would be advisable, with an interval of between 24 and 35 days (minimum 24 for last pupation, maximum 22+13 for first appearance of second generation).

SUMMARY

Observations have been made on the distribution of adult and pupal keds over the body of sheep and lambs, and the seasonal changes in density and distribution have been followed by a series of counts on the same sixteen sheep throughout a period of 13 months.

The method of assessing the adult population by counting the living keds was checked by killing and subsequently counting them, and is believed to give approximately correct results. Pupal estimations by the 'live-count' method gave results apparently 50 % below the actual values.

The ked population builds up rapidly during late winter and early spring to a maximum in April and early May. A decline begins in May, and by June the population has decreased very considerably. During summer the unshorn lambs show a continued decrease, which may be due in whole or part to loss by transference to the shorn sheep.

The greatest concentration during the peak infestation is on the chest, lower shoulder and lower rib wool. In winter and spring the population is more generally distributed, and may show a preference for the upper over the lower side wool. In summer the back is practically deserted and the upper sides only sparsely populated. The throat and chest region is a favoured site the year round, and there is some indication that the throat may be a breeding area.

Generally speaking, the adult and pupal populations are proportionately distributed. In late spring, however, puparia tend to be laid to a greater extent, relative to the adult population, in the lower than the upper surface fleece.

The significance of the seasonal population shifts is discussed, and a method of eradicating keds from a flock by summer dipping of the lambs is outlined.

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AN ESSENTIALLY SYNTHETIC LIQUID MEDIUM FOR *ENTAMOEBA HISTOLYTICA**

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Studies of the nutritional requirements of Protozoa have been confined mainly to free-living ciliates and have seldom dealt with parasitic forms. In order to begin a study of the nutritional requirements of a parasitic protozoan and thus extend the work done with free-living forms, an attempt has been made to develop a synthetic medium adequate for the growth and reproduction of *Entamoeba histolytica*. The initial phase in the development of this medium has been reported (Emerson & Hansen, 1946).

dried liver and proteose-peptone† in this laboratory in 1945, and has been used continuously since June 1945 to carry the stock cultures. This medium has the advantage of being easily prepared and is more satisfactory for the screening of potential amoebicides than is the slope medium. In the development of a synthetic medium, the plan of attack was to replace proteose-peptone and liver with synthetic amino acids and vitamins of the B-complex. The components of the synthetic medium in relation to media from natural products are shown in Table 1.

Table 1. *Nutritional requirements* of* Entamoeba histolytica†

Protein	Nitrogenous bases	Vitamins	Carbohydrate and sterol
SOLID/LIQUID MEDIA			
Egg slope, or agar containing liver, overlaid by saline containing serum or egg albumin‡			Rice starch
LIQUID MEDIA FROM NATURAL PRODUCTS			
Dehydrated proteose peptone		Enzyme digest of liver	Rice-starch cholesterol
ESSENTIALLY SYNTHETIC LIQUID MEDIA			
Twelve amino acids	Nucleic acid	Ten synthetic vitamins of B complex	Rice-starch cholesterol

* Criterion of an adequate medium is maintenance and multiplication of amoebae for eight or more transplants.
† In association with organism *t*, isolated by Dr C. W. Rees, National Institute of Health, Bethesda, Md.
‡ Reardon & Rees, in 1939, found that saline alone was a satisfactory overlay.

The traditional medium for the cultivation of *E. histolytica* consists of a solid slope overlaid by liquid (Dobell & Laidlaw, 1926). More recently, media have been developed in which the coagulated solid portion has been omitted (Balamuth & Sandza, 1944; Chang, 1945; Hansen, 1945; Hitchcock & Rawson, 1946). Such a medium was prepared from

The composition of an essentially synthetic medium is given in Table 2.

E. histolytica and organism *t* have been growing in this medium since 5 March 1946. Cultures were carried over from an earlier and less satisfactory synthetic medium started on 22 December 1945. Transfers were made at intervals of 4 days when the number of organisms reached a peak. Cultures remained viable for at least 8 days. The maximum

* The work was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development, and the University of California, and subsequently supported by the United States Army Medical Research and Development Board.

† Proteose peptone, 2 g.; liver (Wilson's Fraction L-enzymatic digest), 0·3 g.; *l*-cysteine, 0·1 g.; methionine, 0·02 g.; cholesterol, 0·004 g.; buffered saline, 100 ml.

Table 2. *Composition of an essentially synthetic medium*

(Quantities for 100 ml. total.)

Trace minerals	
	μg.
$MgSO_4.7H_2O$	10
$CaCl_2.2H_2O$	10
$FeCl_3.6H_2O$	125
$MnCl_2.4H_2O$	5
$ZnCl_2$	5
Buffered saline	
	g.
NaCl	0·5
$Na_2HPO_4.7H_2O$	1·0
KH_2PO_4	0·1
Amino acids	
	mg.
l-(+)-Lysine HCl	5
l-(−)-Histidine $HCl H_2O$	5
l-(−)-Leucine	5
l-(−)-Tryptophane	5
l-(+)-Arginine HCl	5
l-(+)-Cysteine $HCl H_2O$	5
*d.l-Iso*leucine	10
d.l-Methionine	10
d.l-Phenylalanine	10
d.l-Valine	10
d.l-Serine	10
Glycine	10
Vitamins	
	μg.
Thiamine*	20
Riboflavin*	40
Calcium pantothenate	40
Nicotinic acid	40
Pyridoxin HCl	80
p-Aminobenzoic acid	40
i-Inositol	40
Choline	80
Lactobacillus casei factor†	54
Biotin	2
Other nutrients	
	mg.
Nucleic acid‡	5·0
Cholesterol sodium sulphate§	0·1
Cholesterol	4·0
Rice powder—Difco	100·0 (approx.)

* Thiamine and riboflavin may be completely inactivated during preparation.

† Crystalline fraction; obtained from the Lederle Laboratories.

‡ From yeast; obtained from the Schwarz Laboratories.

§ Recommended by Dr C. W. Rees.

usually was as high as found in natural media, i.e. 400,000 amoebae per culture tube. This number could be reached from an inoculum containing from 3000 to 150,000. Each inoculum was carried over in 0·25–0·5 ml. of the sediment from the mother culture tube. The possibility was considered that survival of amoebae in the synthetic medium might be due to 'adaptation' to a lower nutritional environment. However, new cultures in synthetic medium, begun from stock cultures, persisted at similar population levels. Transfer of a culture carried for months in synthetic media back to media from natural products, resulted in little if any increase in number.

PREPARATION OF MEDIUM

Stock solutions of buffered saline, trace minerals and amino acids were prepared at 10 times the required concentration. The solution of vitamins was made at 50 times the required concentration, cholesterol-sodium sulphate at 1000 times, *L. casei* factor at 400 times and nucleic acid at 50 times. Appropriate volumes of the stock solutions were taken and made up to the required volume of medium with distilled water.

The solid cholesterol was distributed by shaking during tubing of the medium (4·5 ml. per tube). The medium was sterilized by autoclaving at 120°C. for 7 min. The tubes were stored at refrigerator temperature to reduce evaporation. Sterile rice powder was added from a dispenser at the time of inoculation. The *p*H of the sterilized medium was 7·48; this fell to 7·1–7·2 in 4-day-old cultures.

CONSTITUENTS OF MEDIUM

The saline provided a high buffering capacity against the acid produced by organism *t*. Amoebae multiply over a *p*H range of 6·1–7·9 and survive within the wider limits, *p*H 5·4–8·1. The satisfactory range of *p*H for organism *t* is still wider.

With the possible exception of iron, requirements for trace minerals were not determined. The addition of iron as haeme (suggested by the amoeba's ingestion of red blood cells), improved the nutrient properties of the medium. The effect of the accompanying porphyrin fraction was not determined. An attempt to substitute haeme for starch granules was not successful.

Vitamins of the B complex appeared sufficient to maintain growth. In their absence the number of amoebae in culture decreased and disappeared by the fourth transfer. The vitamin requirements were estimated from the reports of Rees, Bozicevich, Reardon & Daft (1944), and by analogy with media developed for the cultivation of bacteria (Knight, 1945). Trials with varying amounts were made only with *L. casei* factor and with biotin. An increase

in *L. casei* factor from 4 to 55 μg. % improved the nutrient properties. Apparently the requirement of amoebae for this vitamin is higher than required by *Tetrahymena geleii* (Kidder & Fuller, 1946). No improvement followed a further increase to 110 μg. %. *L. casei* factor as the sole vitamin, 50 μg. %, was not adequate. A 10-fold increase in biotin did not improve growth.

Cholesterol was included in the same form as found satisfactory in the liver-proteose-peptone medium (see also Snyder & Meleney, 1943).

Preliminary trials showed that nucleic acid probably could be replaced by the four nucleotides, adenylic acid, cytidylic acid, ammonium uridylate and ammonium guanylate, each at a concentration of 1·25 mg. %. This medium was more satisfactory than one in which nucleic acid was replaced by the five bases: *l*-asparagine hydrate at 20, glutamine at 4, and adenine sulphate, uracil and xanthine each at 2 mg. %. Thymine, 10 μg. %, was included in some media. Omission of nitrogenous bases resulted in an unsatisfactory medium.

In addition to the amino acids reported essential for the ciliate, *T. geleii*, by Kidder & Dewey (1945) (with the exception of threonine), glycine and cysteine were used. A comparison with the amino acids essential for man indicates that all are included with the exception of threonine.

The amounts of each were adjusted to provide 5 mg. % of the biologically active form. If both the *d* and *l* forms of methionine and phenylalanine are utilized by the amoeba as in vertebrate nutrition, then the biologically active quantities of these amino acids have been inadvertently doubled.

In another medium the amino acids were included at the levels used by Kidder & Dewey (1945). However, the growth of amoebae did not improve. Nor did any improvement follow the inclusion of other amino acids, viz.: *l*-(−)-tyrosine, *dl*-alanine, *dl*-aspartic acid, *l*-(−)-proline, *l*-(−)-hydroxyproline, *dl*-norleucine, *l*-(−)-threonine and *l*-(+)-glutamic acid. The use of glycine, 20 and 100 mg. %, as the sole amino acid resulted in death of the amoebae in the second transfer. With cysteine and methionine both at 20 mg. %, the cultures died by the fifth transfer.

An opportunity to test the essential requirement for *iso*leucine was inadvertently provided when the supply of this amino acid was exhausted. Cultures in which the level was reduced from 10 mg. to 6 mg. % decreased in numbers. In the complete absence of *iso*leucine a further decrease was noted.

Originally, each of the amino acids was used at a level of 100 mg. %. Such concentration proved toxic for the amoebae. This effect could have been due partly to the low *p*H, 6·25, which approached an unfavourable range in natural media. The reduced quantities (20 mg. %) of amino acids later used were derived from comparison with the content of free amino acid in the proteose-peptone of the liver-proteose-peptone medium. In subsequent modifications, the total amino-acid content was reduced further by lowering both the quantity and number of amino acids.

The toxicity following inclusion of an excess of amino acids and the consequent high acidity might explain the toxic effect of casein hydrolysate (acid hydrolysed, General Biochemicals). Supplemented with tryptophane, this was the source of amino acids in the first media prepared. The levels used were 4, 2 and 0·5 g. %. The toxic effect was less noticeable with the lowest amount employed.

An increase in the amount of cysteine and methionine to the level used in the liver-proteose-peptone medium did not improve growth. It is believed that these amino acids are important in the maintenance of a high reduction potential in the medium. However, there is no precise information about the actual amounts required. If it proves possible to cultivate amoebae without bacteria, it is likely that the amounts of cysteine and methionine will be more critical. The reduction potential required under such circumstances must be maintained either by amino acids or by other means (see also Chang, 1946).

ADDITION OF NON-SYNTHETIC INGREDIENTS

The inclusion of rice powder is anomalous in the attempt to develop a totally synthetic medium. However, it has been found impossible to omit rice powder in any medium (including those from natural products) thus far employed. Replacement with other carbohydrates resulted in excess acidity from fermentation by organism *t*. An attempt to substitute inositol for carbohydrate proved unsatisfactory. It should be noted, however, that rice starch is not itself an adequate nutrient. It is possible that multiplication of the amoebae is directly related to ingestion of rice granules. It is believed that starch serves not only as a source of carbohydrate but also as an adsorbent for other ingredients.

The presence of organism *t* in culture provides a possibility that nutrients elaborated by this bacterium are made available to the amoebae. However, in many media in which they failed to survive, organism *t* was easily maintained. This would indicate that such nutrients are not adequate for maintaining growth of the amoebae.

The critical requirements of each of the ingredients are now under study. The effects of biologic antagonists on the growth and multiplication of *E. histolytica* are our primary concern. When critical levels have been established, chemical relatives of essential substances may be investigated.

SUMMARY

An essentially synthetic medium has been developed for the cultivation of *Entamoeba histolytica* in association with organism *t*. It consists of buffered saline solution containing trace minerals, twelve amino acids, ten synthetic vitamins of the B complex, nucleic acid, cholesterol and rice starch. Cultures have been carried continuously in this medium for a period of eleven months.

We are greatly indebted for materials used in this study to the following: Drs M. S. Dunn, J. J. Eiler, D. M. Greenberg, L. D. Greenberg, E. E. Howe, H. D. Lightbody, H. S. Loring, H. Molitor, E. D. Stewart and E. L. R. Stokstad.

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ON THE GENUS *DINOBOTHRIUM* VAN BENEDEN (CESTODA), WITH A DESCRIPTION OF TWO NEW SPECIES FROM SHARKS, AND A NOTE ON *MONORYGMA* SP. FROM THE ELECTRIC RAY

By NORA G. SPROSTON, *From the Marine Biological Laboratory, Plymouth*

(With 25 Figures in the Text)

Among the tetraphyllidean cestodes of the family Phyllobothriidae, it now appears that a group of four species stands apart and confirms the validity of the genus *Dinobothrium* van Beneden, 1889. Three of these species were recently found in large sharks caught near Plymouth. Two of them are new and a third is new to Britain. The fourth was redescribed from local material by Woodland in 1927.

On 2 November 1945, a young male blue shark—*Carcharinus glaucus* (Linn.)—about 1·5 m. long, was caught offshore and examined here the same day. No copepods or trematodes were found on the skin, gills or other parts of the body, and the liver appeared to be healthy. Apart from a few larval nematodes in the stomach and spiral intestine, the only parasites were three cestodes: two young scolices and a mature strobilus of the same species, all occurring in the spiral intestine. The food remains consisted of four small teleosts (probably *Ammodytes* sp.) and the jaws, setae and other fragments of polychaetes. The cestode, presently to be described for the first time, is a veritable giant in this subclass, having a massive, squarish, scolex which in the fresh state measured just over 8 × 10 mm. It is named *Dinobothrium keilini* n.sp., in honour of Prof. D. Keilin, F.R.S., to whom the author would like to express her gratitude for advice and encouragement in all aspects of her work.

On 8 June 1946, a medium-sized basking shark—*Cetorhinus maximus* (Gunner)—was landed at Cawsand Bay, just west of Plymouth Sound, by Mr Sam White. It had become entangled overnight in his herring-nets, which it ruined; repeating a similar experience he had in 1938, when the only two basking sharks entering the Bay came into his nets. While this shark was being landed, another about the same size was seen a mile from the shore; and during the same week reports came in from fishermen that several losses had been sustained, due to these sharks, at various places along the coast of south Cornwall. Our thanks are due to Mr Sam White, the Cawsand postman, for notifying us promptly and putting this interesting material at our disposal.

The obvious difficulties in the way of examining the viscera of large sharks while still fresh accounts for the unsatisfactory state of our knowledge of their internal parasites and their biological relationships with their hosts. Some notes made while dissecting this specimen are therefore included here for their possible interest in view of the ecology of the parasitic fauna.

This was a young female, 17½ ft. long (5·33 m.), with a girth in the pectoral region of about 8 ft. Any parasites which might have been on the skin would have been rubbed off as the animal was dragged across the beach. The colour was a uniform steely black, shading to only a slightly paler colour below. The thick lips nearly concealed the small, smooth, conical teeth, and the white buccal membrane was thrown into thick triangular papillae. The grey-edged gills were close-set and barely 1 cm. wide, but they were about 20 cm. long. The fine black gill-rakers averaged about 7 cm. long and were set only 0·5 mm. apart, forming a very efficient strainer for the finer plankton. The stomach, the size of a pillow, contained a dryish orange-red paste consisting of the following planktonic organisms: *Oithona* sp. (by far the most abundant), *Pseudocalanus* sp. and *Calanus* sp. (both very common), fish eggs (common—including those of *Trigla* sp.), remains of decapod larvae and other small planktonic organisms. That these specimens appeared fairly fresh indicates an enormous turn-over in the feeding of these sharks: depleting a fishing ground of its plankton stock to an appreciable extent, particularly when it is remembered that they are not solitary in their habits. The spiral intestine was nearly as wide and as long as the stomach, and had a large number of internal coils which were no more than 2 cm. apart; the whole organ was filled with a thick pale chyle. By far the largest organ in the body was the liver; it appeared healthy and was estimated to have weighed some 2 cwt. The gall-bladder was also healthy; it was enclosed in a thick white tunica, adherent to the wall of the duodenum.

When van Beneden examined three large basking sharks (1871, p. 7) he found no parasites, and remarked that none had been found by previous investigators. Since then, cestodes similar to *Dinobothrium septaria* van Beneden (from the

porbeagle, *Lamna cornubica*), with large squarish scolices, have been found in this host by Mola (1906), Masi (1912), Nybelin (1914), Linton (1922), Legendre (1923) and by the present author. It is now clear that these belong to a distinct species apparently restricted to this host—*Dinobothrium planum* Lint.

Legendre also found Copepoda on the skin—*Dinemoura producta* (Müller, 1785)—a species commonly found on other large sharks of the family Lamnidae, and previously recorded from this host in the Mediterranean, and also from the Clyde by A. Scott (1929). Several authors have recorded a dichelesthiid copepod of the genus *Nemesis* Risso, from the gills, and Legendre's material from Concarneau has been admirably redescribed by Fage (1923). Twelve *N. lamna* Risso, 1826, were found on the gills of the Cawsand shark; there were five females with egg-strings, and five younger females but only two males. All these copepods were found clinging to the narrow gills, at about mid-gill level, each with its head pointing to the top of the gill; that is, *in situ* they would be orientated parallel to the long axis of the shark, and thus receive the main current over the gills head-on.

For the first time, a trematode was recorded from *Cetorhinus maximus*: two specimens only, of a blood-fluke belonging to the family Spirorchidae, were recovered from the branchial blood vessels. They represent a genus apparently new to science, and are more closely related to the *Spirorchis* spp. from fresh-water turtles than to the *Aporocotyle* spp. inhabiting the branchial vessels of teleosts; these worms will be described elsewhere.

The stomach yielded no parasites, and it is interesting to note that the six large cestodes—*Dinobothrium planum*—were found exclusively in the duodenum, or proximal intestine, and in the most anterior region of the spiral valve. Previous records have not included the exact site, usually referring them to 'the spiral valve', though Legendre said that they were emerging from the cloaca when he examined his shark; this may have been the result of post-mortem emptying of the gut, but our shark had been dead only a few hours when the viscera were brought to the laboratory for examination. Free in the lumen of the spiral valve two larval nematodes were found, belonging to the subfamily Anisakinae; this kind of larva is exceedingly common in teleosts, and earlier larval stages have been found in calanoid copepods and in *Sagitta* (see Marshall, Nicholls & Orr, 1934, pp. 812–13, and Lebour, 1917, pp. 201–6, respectively), so that it is not surprising to find them in this plankton-feeding shark.

In the middle region of the spiral intestine three specimens of a minute tetraphyllid cestode were found, one, at least, mature. They prove to be a new species, also belonging to the genus *Dinobothrium*, and because of their discovery during the week-end of the official 'V.E.' celebrations, the species is optimistically named *D. paciferum* n.sp. (=*pax fero: pacifer*—peace-bringing).

The recent findings of Dollfus (1936, pp. 523–5) leave no doubt that the intermediate hosts of *D. septaria* are cephalopods of the family Ommatostrephidae; it is reasonably certain that *D. planum* and *D. paciferum* of *Cetorhinus maximus* have a different and very much smaller vector. Cuttle beaks have never been found in its stomach, though it is perhaps possible that this shark could capture a small sepiolid, such as *Heterosepiola atlantica* (d'Orbigny).

Torpedo nobiliana Bonaparte, the electric ray, is not common on these coasts, and for the present material we are indebted to Mr E. Close of West Looe, Cornwall, who secured the specimen while fishing off Plymouth on 28 May 1945. This was a spent female of rather emaciated appearance, measuring 104 cm. long and 71 cm. across the disk. Though all organs were carefully examined in the fresh state, only one internal parasite was found—a single immature phyllobothriid in the spiral intestine, presently to be described. The viscera were healthy and the liver (with the gall-bladder) weighed 2740 g.; there was no trace of food in the gut, but it had swallowed a full-sized *Squalus acanthias* Linn., used to bait the line on which it was taken. The gills harboured three specimens of a monogenetic trematode, probably *Amphibdelloides maccallumi* (Johnston & Tiegs, 1922) Price, 1937.

THE GENUS *DINOBOTHRIUM* VAN BENEDEN, 1889

For the sake of clarity, it is proposed to refer to certain important features of the two known species of this genus, and to set out their revised synonymies before describing the two new species. The genus now comprises: *Dinobothrium septaria* van Beneden (genotype); *D. planum* Linton; *D. keilini* n.sp.; and *D. paciferum* n.sp.

Dinobothrium septaria van Beneden, 1889

D. septaria van Beneden, 1889, pp. 69–70, 71, Pl. figs. 1–3 (immature strobilus from *Lamna cornubica*—Belgian coast).

Thysanocephalum sp. (*T. crispum* Lint.?), of Linton 1897, 792, Pl. LXII, figs. 10–11 (larval scolex from *Ommatostrephus illecebrosus* Verrill—Woods Hole, Mass.).

D. septaria van Beneden, of Lönnberg 1892, pp. 20–4, Pl.; 1898, pp. 19–21, Pl. fig. 10 (immature strobili from *Lamna cornubica*—Norwegian coast).

[non *D. septaria* van Beneden, of Mola 1907 (see *D. planum* Lint.).]

D. septaria van Beneden, of Th. Scott 1909, p. 84, Pl. VI, fig. 4 (young strobilus from *Lamna cornubica*—North Sea).

[(?) non *D. septaria* van Beneden, of Masi 1912 (see *D. planum* Lint.).]

[non *D. septaria* van Beneden, of Nybelin 1914 (see *D. planum* Lint.).]

D. plicitum Linton, 1922, pp. 2–5, Pl. I, fig. 1, Pl. II, figs. 4–6 (immature strobili from *Carcharodon carcharias* (Linn.)—Woods Hole).

[(?) non *D. plicitum* of Joyeux, in Legendre 1923 (see *D. planum* Lint.).]

Phyllobothrium septaria (van Beneden), of Southwell 1925—*partim*—pp. 169–70, figs. 99–100 (copies of v. Beneden, fig. 2, and Linton's fig. 1 respectively).

D. septaria van Beneden, of Woodland 1927*a*, pp. 231–40, 245–6, Pl. XII, figs. 1–10, Pl. XIII, figs. 11–13 (mature strobili from *Lamna cornubica*—Plymouth).

D. septaria van Beneden, of Joyeux & Baer 1936—*partim*—pp. 94–5, fig. 40 (copy of Linton's fig. 1).

D. plicitum Linton, of Dollfus, in Joyeux & Baer 1936, pp. 523–6, figs. 557A–E (larval scolices from *Todaropsis eblanae* (R. Ball)—Concarneau, France).

Woodland's description is the fullest and the first dealing with gravid proglottides; he gives a review of the previous records and maintains, with Southwell, the identity of Linton's *D. plicitum* with the genotype, but differs from him in retaining the genus as separate from *Phyllobothrium* van Beneden, 1849, and also in preserving the validity of *D. planum* Linton. In this the author is in agreement with Woodland; but some of his criticisms of previous descriptions are partly nullified because he failed to realize (through lack of comparative material) that the European authors had been describing two distinct species under the name of the genotype.

The characteristic feature of the scolex of *D. septaria*, evident even in larval forms from cephalopods (see Dollfus, 1936, fig. 557 B–E), is that the double roof-like ridge joining the anterior ends of the bothria pairs has its sharp lower edge cleft in the median line so that two adjacent triangular prongs extend downwards over the edges of the bothria (Linton, 1922, fig. 1 and Woodland, 1927*a*, fig. 1). The outer ends of the anterior ridge, as in all species, are curved inwards and downwards with a twist through nearly a right angle, ending in a stiff bifid lobe with broad points—fully visible only in profile view (Figs. 1–2 herein). A small sucker-like organ occurs on the shelving portion of the ridge above each bothrium, slightly medial to its centre line (situated much as in *D. planum* and *D. keilini*, where the structure is a true sucker—Figs. 3 and 12 herein); but Woodland states that in *D. septaria* the constituent fibres run at right angles to the surface, so that it can only be described as a 'dimpled swelling' on the ridge (Linton, 1922, fig. 4; Woodland, 1927*a*, figs. 1–3). The bothria have thin edges and tend to become sharply pointed distally, the point being really double, since it is the end of a furrow extending halfway up the middle of the bothrium. This feature has not been seen in the other species, and it is figured only for some of the larger scolices of *D. septaria* (Linton, 1922, fig. 1; Scott, 1909, fig. 4). The hind ends of the bothria appear to be free for about half their length, and are slightly flared anteriorly, and they are deeply scoop-shaped—fide Linton.

There is no neck in this species, and it is curious that Woodland did not mention the peculiar ventral furrow running down the middle of the nascent proglottides, so conspicuous in Linton's fig. 1. Woodland's specimen, though only 110 mm. long, had ten to twelve gravid proglottides at the end which were longer than wide (3·5 × 1·5 and 3·0 × 2·0 mm.) whereas Lönnberg's strobilus was 180 mm. long, and the 'immature' (mature, but not gravid) segments were some three times as wide as they were long (1898, p. 19 and fig. 10). Woodland (1927*a*, p. 232) suggests that van Beneden erred in stating that the type specimen was 25–30 mm. long, and that it was more likely to have been 120–30 mm. On comparing his drawings with the measurements of recorded scolices, and with Scott's photograph (1909, fig. 4), it appears that van Beneden's fig. 1 is natural size; I have measured this drawing and find it to be 265 mm. long, and the terminal proglottis is practically square, yet he says, immature. I think it is possible to explain this discrepancy in the length of the different strobili in front of the region bearing gravid proglottides. In most of my specimens of *D. planum* there is, in the accepted sense, no 'neck' and narrow, ventrally grooved, incipient proglottids appear immediately behind the scolex. In some of them there is a region a short distance behind the scolex which appears completely unsegmented, and this region extends for 72 mm. in one of them. It is to be noted that such a region is narrower than that immediately in front; but when it is remembered that the predominant tissue in this part of the strobilus is longitudinal muscle, it is not necessary to attribute such an extension, obliterating the traces of the nascent proglottides, exclusively to post-mortem relaxation. It may occur in life to compensate for the sharp coiling of the spiral valve, and its peristaltic movements near the duodenum, where these worms were found. Fixed strobili including such elongated 'pseudo-necks' would have a misleading total length (see p. 77 below). The occurrence of an ephemeral 'pseudo-neck' in these cestodes may therefore be an adaptation of the relatively very large body of the parasite, to the peculiar set of strains sustained by it in the elasmobranch gut. In contrast with these species, it will be recalled that most of the tetraphyllidean cestodes in elasmobranchs are minute forms, and capable of being accommodated in, at most, a single turn of the spiral intestine. Cestodes of teleosts and mammals, on the other hand, inhabiting a gut with few abrupt twists, are relatively large forms.

Woodland demonstrates a peculiarity in the excretory system of *D. septaria*: the longitudinal excretory vessels open to the exterior, at the hind end of each mature proglottis, into an open 'bay' which overhangs the narrower end of the succeeding proglottis. This was also noted in the present material of *D. planum* and of *D. keilini*, where the transverse communicating vessel has apparently become atrophied. His descriptions of the genitalia agree with those of Lönnberg (1898) except in two particulars; Woodland alone describes a longitudinal split in the ventral body-wall through which the gravid uterus sheds its eggs to the exterior as they become ripe—the gravid proglottides not normally becoming detached from the strobilus. Both authors show the characteristic course of the voluminous vagina, passing from the common genital atrium, in front of the cirrus-sac by many convolutions, to the anterior edge of the proglottis, thence descending down the middle to the posterior end where it enters the fertilization chamber. Woodland correlates the enormous volume of the vagina and its storage capacity with 'a brief but energetic period of sperm production' (1927*a*, p. 236). By this he implies marked protandry, which is an important feature in the economy of space in the cestode proglottis; a device also securing cross-fertilization, to some extent, and certainly protracting the physiological drain of producing both male and female reproductive elements. He does not mention the two sphincters on the dilated part of the vagina lying parallel to the cirrus-sac; Lönnberg described and figured them much as they are in *D. keilini* (Fig. 17 herein). Vitellaria are present in all mature proglottides of this species, situated as two sheets of smallish follicles ($40 \times 26\ \mu$) extending external to the main excretory vessels and nerve laterally, both dorsal and ventral, over the outer thirds of the proglottis (Woodland, 1927*a*, figs. 7–13). The specific characters of this and the other species are summarized below in tabular form (p. 87).

Dinobothrium planum Linton, 1922 (Figs. 1–11)

D. septaria van Beneden, of Mola 1907, pp. 4–6, text-fig. 2, Tav. iii, figs. 5–8, Tav. iv, figs. 12–18 (mature strobili from *Cetorhinus maximus*—Elba).

[?] *D. septaria* van Beneden, of Masi 1912, p. 323 (from *Cetorhinus maximus*—Mediterranean).

D. septaria van Beneden, of Nybelin 1914, pp. 228–31, figs. 1*a–b* (immature strobili from *C. maximus*—North Sea).

D. planum Linton, 1922, pp. 5–8, Pl. I, figs. 2–3, Pl. III, figs. 7–10, Pl. IV, figs. 11–13 (mature strobili from *Cetorhinus maximus*—Woods Hole, Mass.).

[?] *D. plicitum* Linton, of Joyeux, in Legendre 1923, pp. 278–9 (mature strobili from *Cetorhinus maximus*—Concarneau, Finistère, France).

Phyllobothrium septaria (van Beneden), of Southwell 1925—*partim*—pp. 169, 170–1, fig. 101 (copy of Linton's fig. 2).

D. planum Linton, of Woodland 1927, pp. 231, 240.

D. septaria van Beneden, of Joyeux & Baer 1936—*partim*—pp. 94–5.

The present material consists of six strobili from *Cetorhinus maximus*: four of these (measured in the fresh state) were 360, 380, 480, and 560 mm. long; two others, measured after preservation, are 546 and 535 mm. long. Linton's material was about the same length, but one of his specimens was as long as 825 mm. Mola's mature, but not gravid, strobili were up to 120 mm., and those of Nybelin were 130 and 155 mm.

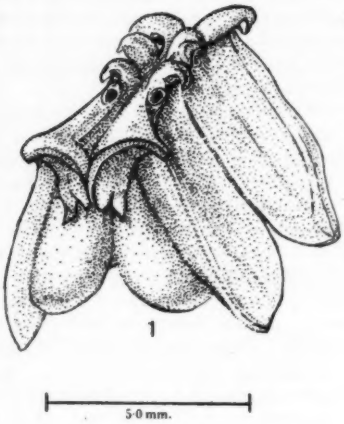
The scolex is slightly larger than that of *D. septaria*: Linton's measurements were between 7 and 8 mm. long and up to 10 mm. across the widest part of the bothria pair; Nybelin's were 7×9 mm.; and Mola's (by measuring his drawings) are $6{\cdot}7 \times 8$ mm., not including the ridge and crest. In the present collection the bothria pairs in the mounted material measure $6{\cdot}3 \times 7{\cdot}7$, $5{\cdot}9 \times 7{\cdot}5$ and $6{\cdot}25 \times 7{\cdot}6$ mm.; all showing a fairly constant ratio of length to maximum width, which in this species occurs in the anterior half of the bothria, whereas in *D. septaria* it tends to be in the posterior half. There is no median furrow in the bothria, and they are shallower and have thicker edges than those of *D. septaria*.

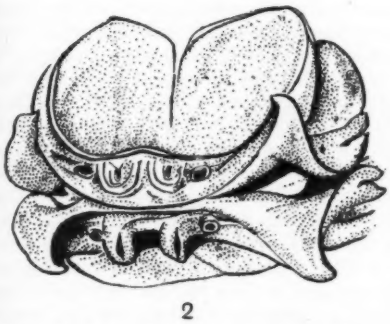
An unusual feature, not mentioned by Linton or others, is the thin bag-like swelling at the back of each bothrium, which when it is distended keeps the bothria flared upwards (Fig. 1). Any pressure applied to the scolex during fixation, or an incision, permanently deflates these organs. They are structurally distinct from the leaf-like region of the bothria, and their inner wall is continuous with that of the central column of the scolex. Their anatomy is difficult to make out because the wall is thin and on deflation becomes collapsed on to the hind face of the bothrium. It is suggested that they may be vesicles in communication with the excretory vessels (which, since they are open, may also have a hydrostatic function); the latter enter the bothria along the line of attachment of the vesicles (cf. the arrangement and course of the vessels in the scolex of *Phyllobothrium dohrnii*, in Rees, 1946, p. 170, fig. 7).

The scolex resembles that of *D. septaria* and *D. keilini* in having a typical anterior ridge, but here it is thicker, and instead of the sharp median prongs directed downwards over the edges of the bothria, there are two erect and slightly curved knobs, bearing a faint longitudinal depression on their outer face (Figs. 1, 2). In the angle between this 'pseudo-sucker' and the shelving ridge, there is a true sucker, with fibres running parallel to its surface and having a thinner outer lip; this is in marked contrast to the 'pseudo-sucker' in which the fibres

are coarser and run from side to side of the organ, except at its base, where oblique fibres attached to the cuticular margin converge deeper in the scolex behind the bothrium (see Fig. 4).

The reflex bifid lobes at the outer angles of the ridge are characteristic of all species of the genus; their constituent fibres run slightly obliquely from side to side, as indicated in Figs. 3 and 14. The fibres



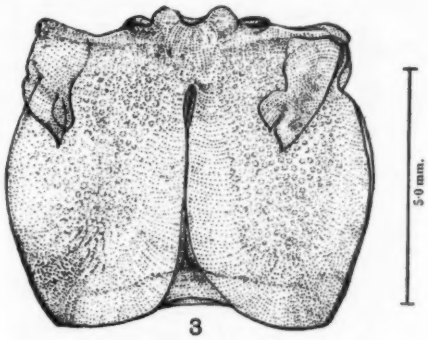


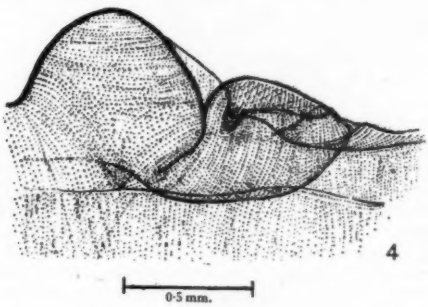
Figs. 1, 2. *D. planum* Lint. Fig. 1. Scolex (fixed without pressure) half-profile view. Fig. 2. The same, viewed from above.

of the bothria are also indicated in the figures by stippling; bundles are attached in the central region and radiate towards the edges (Figs. 3, 4 and 12–14).

In this species the bothria are free from the scolex for about a fifth to a quarter of their length (Fig. 1). There is no true neck, and the ventral furrow down the middle of the nascent proglottides is very conspicuous in contracted specimens; but this and all other incipient structures disappear in regions where a 'pseudo-neck' has developed owing to relaxation of the longitudinal fibres. Two of the specimens were mounted in short lengths on slides, and both of these show about the same length of 'pseudo-neck', though it is rather surprising that measurements of the strobili up to the first fully gravid proglottis should have given the following relatively consistent lengths: specimen A, 397 mm. and specimen B, 378 mm. The length of strobilus before the eggs appear in the uterus was about 260 mm.

Linton remarks on the tendency of the anterior region of the strobilus to develop longitudinal furrows, but he does not pursue the subject. In the

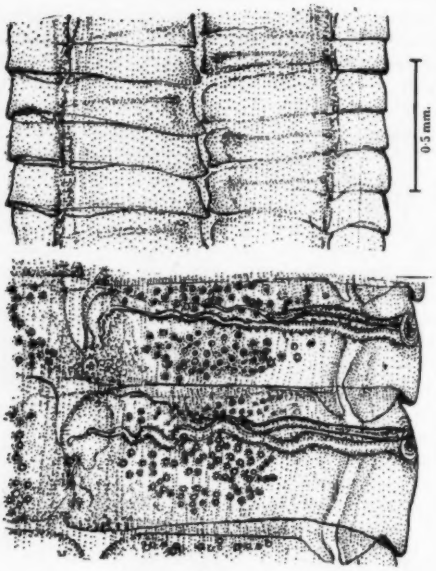




Figs. 3, 4. *D. planum* Lint. Fig. 3. Half-scolex (fixed under slight pressure) viewed from inner face. Fig. 4. The same, a more highly magnified view of the pseudo-sucker and sucker. The direction of the fibres is indicated by the stippled lines.

present specimens of *D. planum*, in the region of the nascent proglottides, the dorsal side is considerably wider than the ventral side, and in partially flattened preparations the dorsal side has a very conspicuous furrow on either side, coming to lie just above what is in reality the margin of the morphologically ventral side (the 'over-lap' being morphologically dorsal). This is represented in Fig. 5—the ventral view of a short series of nascent proglottides—where the morphological edge of the ventral side can be seen on either side as the level reached by the darker transverse streaks, representing the developing genital

ducts. All along these two lines on the dorsal surface there is a break in the body-wall, communicating with the dorsal excretory vessel; the posterior ends of this cleft in each proglottis are quite free as they



Figs. 5, 6. *D. planum* Lint. Fig. 5. Nascent proglottides (ventral view), showing over-hanging dorsal surface on either side. The lateral dorsal furrows, with developing 'pores', over the excretory vessels; ventral excretory vessels opening on to ventral lateral furrows very near to the true ventral margin—marked by the ends of the streaks representing the developing genital ducts. Note the in-turning of the proglottis-halves along the ventral furrow—at this stage an open median cleft. Fig. 6. Dorsal view of just over half of two developing proglottides in the early 'male phase'—the testes are about a third developed, the genital ducts, as yet uncoiled, are about equally developed, and the pores now lie on the margin of the strobilus. The pores over the dorsal excretory vessel have closed, and this now opens to the exterior through a deep notch in the lobed border of the segment. The median ventral furrow is closing at the base of each proglottis in the region of the developing ovarian-complex—the uterus is appearing on the poral side of the open cleft. A fascia of longitudinal muscle fibres is conspicuous, passing from segment to segment.

are in all proglottides, extending downwards as two more or less broad points (see Figs. 5–7). The dorsal cleft is prevented from gaping by four or five bridges of tissue, but in older proglottides these elongated pores become closed, and the cleft becomes a mere notch in the posterior border in mature segments (Figs. 8–11). The ventral 'bay' described by Woodland, as existing in *D. septaria* in the form of an open pocket along the posterior ventral edge of the segments, is not so strongly developed in *D. planum*; here the edges of the segments are lifted and bear a slight notch only, where the opening to the ventral vessel occurs. In whole mounts the dorsal vessel sometimes appears to lie internal to the ventral, and sometimes the latter is not conspicuous, for it is often filled with amorphous material and stains like the parenchyma (Figs. 6, 7).

The ventral furrow of the early part of the strobilus was mentioned casually by Linton for both species, and in *D. planum* he also remarks on (1922, p. 6) '...the development of longitudinal furrows, the most conspicuous being a median furrow on one of the flat surfaces (ventral), especially noticeable in the anterior region. Transverse sections made through unripe sections show a striking waist-like constriction at about the middle, indicating a median furrow on both the dorsal and ventral sides.' He gives no figures of this nor does he pursue the matter, and Woodland does not mention it in *D. septaria*, though Linton has shown it to exist. In the nascent proglottides of both *D. keilini* and *D. planum* it is an open channel, continuous from one proglottis to the next (Figs. 5, 15). The free edges of this cleft have a characteristic shape of interlocking curves, and in spite of being free they do not flare outwards, but bend inwards, forming the furrow which is so conspicuous to the naked eye. The proglottis is therefore thin along this line, and in fixed material a corresponding shrinking-in of the dorsal surface also occurs; this may account for the dorsal 'furrow' and 'waist' effect mentioned by Linton, but there is no actual cleft in the dorsal side like that along the ventral (Fig. 5). Ovarian tissue develops in each of the posterior angles of the cleft wall of the proglottis, and in older regions the development of the ovarian region closes the gap here, leaving, however, a V-shaped hollow in the anterior half of the proglottis (Fig. 6). In maturing regions of the strobilus this is entirely filled by the median pouch of the uterus, which is the first to develop (Fig. 7). In superficial ventral view this appears now as a small dark mound; but it is not until much farther down the strobilus that the gravid uterus is seen bulging prominently through the torn wall of the proglottis, its frayed wall actually liberating eggs to the outside, but the edges are not regularly oval as shown in Woodland's fig. 6 for *D. septaria*. It is now clear that the early development of the ventral furrow is a preparation to accommodate the uterus and to prevent complete rupture of the proglottides, which, as it is, are pre-hinged along five longitudinal lines; thus they can expand in spite of the comparatively rigid

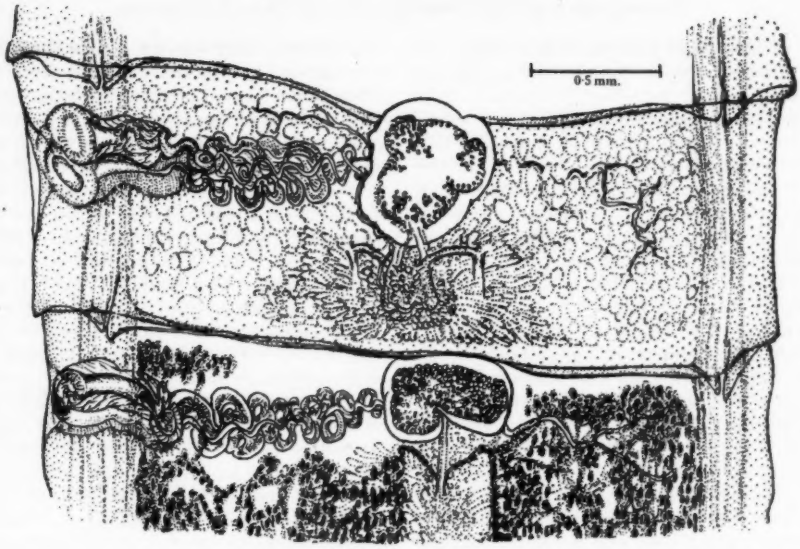
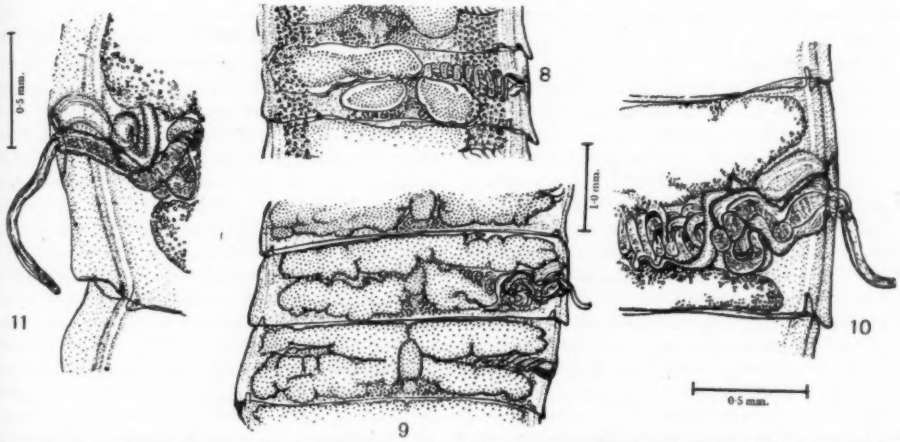


Fig. 7. *D. planum* Lint. Ventral view of two proglottides near the end of the 'male phase' and the beginning of the 'female phase' (these two proglottides occurred as Nos. 6–7 after the one in which eggs were first seen in the uterus—at 257 mm. strobilus-length). In the upper the (waning) testes are shown and the branching of the vas deferentia of either side; the main trunk is distended with sperms, but the vagina in these instances, is not. The developing ovary is shown in both, and the median portion of the uterus is already bulging through the ventral cleft (not shown) in this area of the body-wall. The two main vitelline ducts are indicated, but in the lower proglottis the testes are omitted and the reticulum of vitelline follicles is shown (see text). The clefts giving access to the dorsal excretory vessels are shown (at a deeper focus); and above them there is a slight notch over the ventral 'bay' giving access to the ventral vessels on either side.



Figs. 8–11. *D. planum* Lint. Fig. 8. Ventral view of half-gravid proglottides in which the three main lobes of the uterus are filling with eggs from an active ovary. The vitellaria are receding towards the margins, and the testes are spent and shrunken. Fig. 9. Nearly fully-gravid proglottides with bulging median portion of uterus; vagina and vas deferens distended with sperm. Fig. 10. Dorsal view of the everted cirrus and surrounding organs shown on the right of Fig. 9. Fig. 11. Ventral view of the only other everted cirrus in this strobilus—the spines have been lost from both these cirri. The dorsal excretory vessel alone is shown—the ventral being at a higher focus, but it was not so clear owing to amorphous contents. The lateral nerve is indicated in both figures.

body-wall of this large species, and continue as living members of the stobilus, maintaining egg production.

The testes develop early, and in Fig. 6 they can be seen about one-quarter formed, while the vagina and cirrus-sac are nearly straight tubes. It is seen that the ventral side has grown laterally so that the ends of the genital ducts now abut on the margin, instead of some way in on the ventral side as in Fig. 5. In subsequent proglottides it is interesting to watch the development of the genital ducts, growing in length and twisting on each other as they do so. In mature proglottides they are intimately coiled together, sometimes the one is more prominent, sometimes the other, both usually distended with sperms, the one mass inward-bound and the other outward-bound. At this stage (Fig. 7) the testes show a maximum development; they are oval and larger than those of *D. septaria* ($51 \times 47\ \mu$ fide Woodland) ranging from 75 to 125 μ long, but averaging about $80 \times 50\ \mu$; their number is fairly constant, 270–300 in each proglottis. The cirrus-sac is thick with oblique fibres, and the cirrus is in two parts as described by Woodland for *D. septaria*. The short proximal part (that portion nearer the genital atrium in the retracted state) has larger spines than the long distal part. Only twice was a cirrus found everted (Figs. 10, 11), but the spines had been lost by the time they were mounted for examination, though the lumen was still full of sperms. Evidently the long-coiled vas deferens acts as a vesicula seminalis, for in these proglottides the male phase had long passed, the testes were spent and shrunken (Fig. 9) and the uterus had fully developed both large anterior lobe and posterior lateral lobes. The vagina is much like that described for *D. septaria* and is not quite so voluminous as in that species nor as in *D. keilini*, and unlike these it lacks the prominent sphincters.

The distribution of the vitellaria is unusual in *D. planum*, and Woodland's criticism of Mola (1907) and Linton (1922) was not entirely justified, since both these authors were describing conditions in *D. planum*, which Woodland assumed could not differ in what he considered to be a family character. These authors stated that vitellaria occur only along the ventral side of the proglottis, over which they are continuous; but Woodland maintains that they have been mistaken, and that what they were describing was in reality ovarian tissue. In the present material of *D. planum* the vitellaria are first conspicuous in proglottides in which the testes are fully developed—the end of the 'male phase'—and when the eggs are just beginning to enter the uterus from the uterine duct (Fig. 7). They are brownish in colour and nearer the surface than the long strands of ovarian tissue; the follicles are ellipsoidal islets strung together by fine ducts forming a loose reticulum, but predominantly in longitudinal rows. At their maximum development they extend across the ventral side from inside the lateral nerves and excretory ducts, in the posterior half of each proglottis, as far as the middle third of the ovarian region; here the main vitelline ducts pass forward, that on the aporal side receiving ducts from the anterior group of follicles. On the poral side the follicles are sparse anterior to the vagina and vas deferens, which also interrupts them laterally, though a few chains of follicles pass under these organs, joining the posterior groups with the small antero-lateral group of the poral side. Dorsally on both sides of the proglottis, the reticulum of follicles passes round the outside of the testes, but does not extend for more than a quarter of the width of the segment—usually much less. In older proglottides, the reticulum of vitelline follicles appears to be drawn back from the more central region on the ventral side, and becomes condensed into two rather narrow lateral bands; the individual follicles are now more darkly pigmented (Fig. 8). The dorsal bands have also shrunk laterally, so that they are difficult to see in surface view. Still older proglottides, in which the uterus is fully developed, usually fail to show any vitellaria on either ventral or dorsal surface view. Perhaps the enormous mass of eggs produced in this species accounts for the shrinkage of the vitelline tissue at this stage (as also occurs in *D. paciferum*). The eggs are thin-shelled and they are very nearly spherical; ten ripe eggs had an average diameter of 17·4 μ, being distinctly smaller than those of *D. septaria*, with an average egg size of 25·6 μ.

Dinobothrium keilini n.sp. (Figs. 12–18)

The single mature strobilus recovered from the spiral intestine of *Carcharinus glaucus*, unfortunately became fragmented while it was being removed from the thick chyle and mucus of the middle whorls, and some of the proglottides were lost, so its total length is unknown. A few mature proglottides were recovered in chains, and one had the uterine duct crammed with ripening eggs—in one region some fully formed eggs were found and measured.

The scolex is somewhat larger than our specimens of *D. planum*, and it is altogether more massive in appearance, partly because the bothria are not free at their hind edges. In the fresh state the bothria were held squarely away from the axis of the scolex by the bulbous swellings behind them, similar to those of *D. planum*; but in *D. keilini* the bag-like structures come nearer to the edges of the bothria on the one side, and on the other are more closely applied to the body of the scolex. Fig. 12 is from a scolex which has been slit longitudinally between the pairs of bothria, and the half-scolex is mounted with slight pressure. Before fixation this measured over 8 mm. long and 10 mm. across the widest part. The bothria are conspicuously more rounded than in the

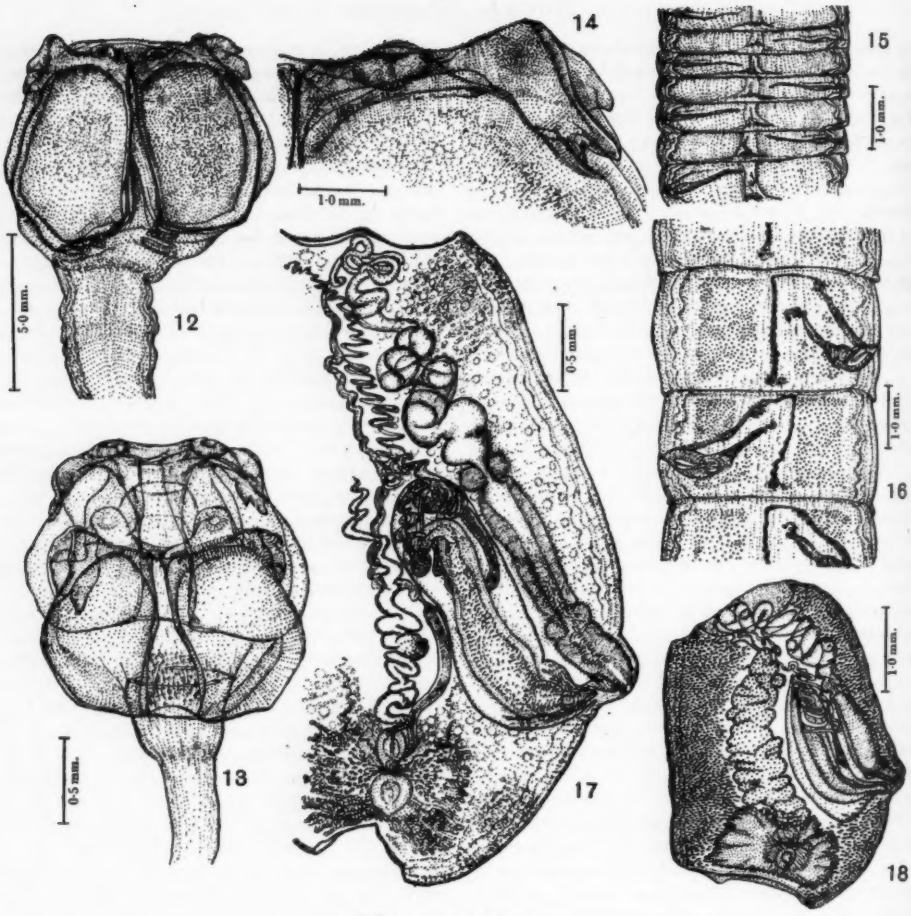
other two species, and the maximum measurements of the fixed bothria are 7 mm. long and 4·3 mm. wide. The anterior ridge is formed of a thick fold of tissue, and it passes into a simple fold continuous with the edge of the bothria; quite unlike the sharp-edged ridges, extending to the two overhanging prongs of *D. septaria*.

The smaller of the two young scolices, found along with the mature strobilus, is shown in its entirety in Fig. 13; during fixation the nearer side was slightly displaced downwards, but this has the merit of separating otherwise superimposed structures and also shows the apical nerve ring. There is, however, no myzorhynchus or other apical prominence in this form, though a first glance at the figure might suggest this. The bothria pairs here measure 1·12 mm. long and 1·3–1·6 mm. across. The older scolex is not figured; its bothria pairs measure 2·15 mm. long and 2·27 mm. across.

The anterior suckers of all specimens are more prominent than the pseudo-suckers of *D. septaria*, and, as is shown in Fig. 14, they are shallow cup-shaped and the fibres are arranged parallel to the surface, as they are in the true suckers of *D. planum*; there are no pseudo-suckers in this species. There is a short but distinct neck in *D. keilini*, and a ventral furrow is present, but it is less marked than in *D. planum*. The nascent proglottides, however, show no sign of the longitudinal furrows on either side associated with the lateral excretory vessels. The vessels themselves are very conspicuous throughout the strobilus as clear wavy bands; anteriorly they can be seen entering the base of the swellings behind the bothria, making about three zigzag bends as they do so (Fig. 12). There is an open 'bay' along the posterior margin of all the segments, though it appears to be very much narrower than that of *D. septaria* described by Woodland. The notches near the openings of the excretory vessels, described here in the posterior border of the proglottides of *D. planum*, are lacking in *D. keilini*, but the excretory ducts can be seen to open into a narrow local uplifting of the proglottis margin, in even the nascent proglottides. It is not possible to say why the device used in *D. planum*—the potential hinges provided by the four lateral furrows over the excretory canals—is not used in *D. keilini*, which is an equally large worm; but it may be that the latter species has a more flexible and muscular body-wall, for longitudinal muscle fibres are very conspicuous, passing over the proglottides in the nascent region, and elsewhere the cuticle appears to be thinner than in *D. planum*.

D. keilini is remarkable in that the cirrus-sac and its internal vesicula seminalis is clearly developed before the vagina has a recognizable wall (Figs. 15, 16), and it is at this stage a darkly staining column of tissue with broader masses indicating incipient convolutions (Fig. 16). From the beginning, as in *D. septaria*, it is directed forwards, and after a few convolutions follows the anterior margin of the proglottis to the midline; from here it descends sharply, and in older proglottides making several convolutions again before its slightly coiled descent (Fig. 17). The descending coils are narrower and pass ventrally to the uterine duct (as in *D. septaria*); at the level of the cirrus-sac it dilates slightly to form the so-called seminal receptacle before entering the fertilization chamber (the whole of the voluminous vagina is in effect a receptaculum seminis). This peculiar course of the vagina is characteristic also of *D. septaria*, and so also (fide Lönnberg) are the two vaginal sphincters. The vagina of *D. keilini* appears to be somewhat larger than that of *D. septaria*, having thicker walls in the dilated terminal region, which are lined with small backwardly directed spines for about half its length.

The cirrus is very powerful and the proximal portion rather longer than that described by Woodland in *D. septaria*. At the outer end of the cirrus-sac is a conspicuous group of gland-cells, not mentioned by any other author for this genus (Fig. 17). In this species the vas deferens outside the cirrus-sac is always narrower than any part of the vagina, its coils are more numerous and it pursues a forward course between the ascending and descending coils of the vagina to near the anterior margin, where it bifurcates and descends, ramifying into smaller sperm ducts. The testes are more numerous than in *D. planum*, but they are about twice the size of those of *D. septaria* (which are spherical); here they are variable in shape and appear to be larger nearer the anterior end of the proglottis. Average measurements from three different levels of the same proglottis (Fig. 17) were 125×87, 92×66 and $92 \times 75\ \mu$. The ovarian complex agrees in almost all particulars with that of *D. septaria*, and is very like that of *D. planum*; the shell-gland is conspicuous against the middle of the large and rather massive ovary. In Fig. 17 the two main vitelline ducts are shown entering the shell-gland, and the distribution of the vitellaria is shown only above and below, and elsewhere diagramatically by the stippled transverse lines. The full development of the vitelline follicles has evidently been reached in the proglottis shown in Fig. 18 (a parallel stage of development to that in *D. planum* of Fig. 7); the follicles are very dark brown and are transversely ellipsoidal, their size range being $21–33 \times 75–58\ \mu$, though some towards the ends of the bands were over $100\ \mu$ wide (Woodland states the maximum size for those of *D. septaria* as $40 \times 26\ \mu$). The most advanced proglottis in our collection is shown in Fig. 18, in which the uterine duct only is distended with eggs, the uterus, presumably occupying the whole of the middle region, being as yet devoid of eggs; a few of those in the uterine duct, however, appear well developed, and measurements of three, undistorted by pressure



Figs. 12–18. *D. keilini* n.sp.

Fig. 12. Scolex from mature strobilus (mounted half-scolex, seen from the outer face; owing to pressure the 'bulbous vesicles' behind the bothria are seen projecting on either side of them).

Fig. 13. Scolex of very young strobilus (whole-mount, nearer half slightly displaced downwards).

Fig. 14. Bothridial crest (portion of half-scolex, mounted with pressure) showing true sucker and reflexed bifid lobe, here folded, at the outer end of crest. Direction of muscle fibres, as in other figures, shown by stippled lines.

Fig. 15. Nascent proglottides (*cf.* Fig. 5). Note developing genital ducts and wide ventral cleft, here of different shape on the poral and aporal sides of the proglottis. The ventral excretory vessel in this species is throughout the strobilus much wider and more conspicuous than the dorsal; the inter-segmental openings of these vessels are never so obvious as in *D. planum*, and are not accompanied by furrows or notches in the body-wall.

Fig. 16. Young proglottides (*cf.* similar stage in *D. planum* Fig. 6). The numerous testes are at an early stage, but no vitellaria could be seen. Note the comparatively early development of the median region of the vagina in this and previous Fig., and yet the development of its distal portion lags far behind that of the cirrus-sac, which is more advanced than in Fig. 6. The fascia of longitudinal muscle fibres obliterated the ventral cleft from this dorsal view.

Fig. 17. Dorsal view of portion of maturing proglottis at the beginning of the female phase: the vagina is distended with sperm and eggs are beginning to fill the uterine duct. Note the two sphincters on the vagina and the fine spines in its distal portion.

Fig. 18. Dorsal view of mature, but not gravid, proglottis showing maximum development of vitellaria, and ovary with medial shell-gland surrounding the oötype and fertilization chamber above; followed by the now very distended uterine duct, packed with developing eggs; the underlying uterus, destined to fill the median field, is as yet empty.

of their neighbours, are: 27·5 × 22·5, 31·5 × 21 and 32 × 19 μ.

The mature proglottides have a very characteristic barrel-shape on one side due to the protrusion of the powerful terminal genitalia, no hint of which is shown in the figures of van Beneden and Woodland for *D. septaria*. Though *D. keilini* is in many ways very close to the genotype, the character of the scolex is quite distinctive, and taken together with the characters of the vagina and cirrus and the larger testes, vitellaria and eggs, there can be no doubt that it constitutes a new and valid species.

Dinobothrium paciferum n.sp. (Figs. 19–23)

This distinctive species from the middle region of the spiral intestine of *Cetorhinus maximus*, has a scolex with facies of *Dinobothrium* van Beneden, and, in addition, certain unique features which may later prove to have generic significance; moreover, it is only about a tenth the size of the three other species of this genus.

The three specimens recovered were all of similar size in the scolex, but in different stages of development; one of intermediate length was unfortunately lost during the process of clearing it, but two remaining (syntypes) are figured here *in toto* (Fig. 19). They must have been dead for some little time when found, as they were extremely fragile and flaccid, which accounts for the probably unduly elongated neck of the more advanced specimen, and the collapse of the scolex of the other as it was transferred to the slide for fixation. The total length of these specimens is 17·2 and 8·1 mm. respectively. The over-all measurement of the scolex in both directions is 0·65 mm.

The scolex (Fig. 20) has two pairs of very thin, slightly scooped, salient bothria. Their long pendant ends extend for half their total length beyond their line of attachment to the body of the scolex. The muscular tissue of which they are composed is so tenuous that it is difficult to make out the anterior region of the bothria, but it appears that the nearly circular area is not an incipient sucker as in *Calyptrobothrium* (*Phyllobothrium*) *riggii* Monticelli, 1893, but the inner edges of the initial fold of the bothrium over its greatest concavity. (In Fig. 20 these areas have been drawn with a sharper definition than they actually possess, a device used to prevent their

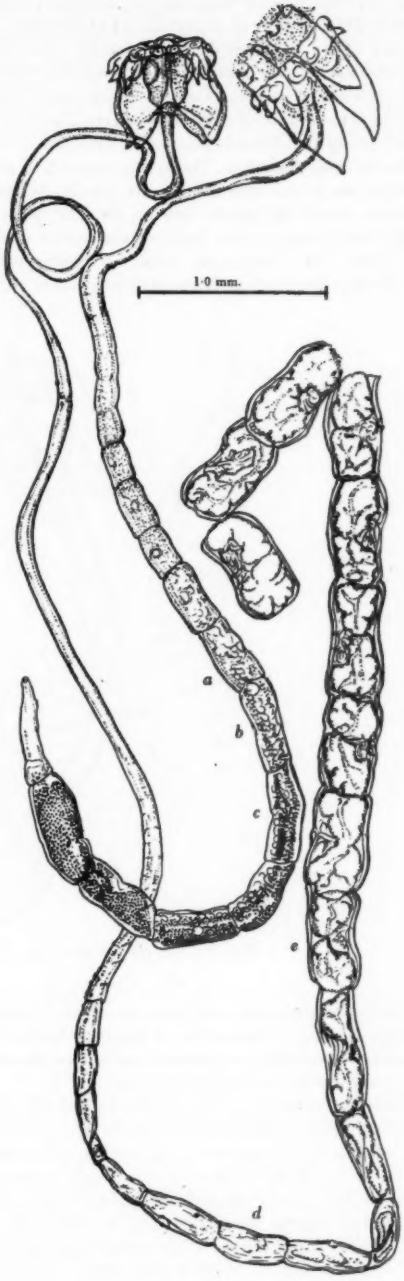


Fig. 19

Fig. 19. *D. paciferum* n.sp. Whole mount of both syntypes—the scolex of the younger strobilus had begun to disintegrate when fixed, and its halves are displaced, and for the same reason the 'neck' of the other specimen is probably unduly elongated. Vitellaria in the posterior proglottides of the younger specimen completely obscure the other structures. (The letters *a–e* indicate regions illustrated in higher magnifications in Figs. 21–23).

confusion with underlying structures shown by transparency.) The anterior ridge is rigid, but not a separate fold of tissue as in *D. septaria* and *D. planum*, but it bears at its outer ends the pointed bifid lobes characteristic of the genus. The outstanding feature is the presence of two true suckers on the crest of each bothrium: their lack of uniformity in the figure is due to their being in different states of contraction—itself a proof of their function. There is a suggestion of an apical organ in this species, but no special adhesive structure could be made out on it. The pairs of bothria are joined to the body of the scolex and to each other by a separate band of tissue which extends fanwise into the back of their posterior half.

striking feature of some of the mature proglottides, particularly those in the hind region of the shorter strobilus. In their extreme development the vitellaria extend from the lateral fields right across the proglottis on both sides, always leaving a clear area round the genital pore. The proglottides are nearly cylindrical in this species; the cuticle is relatively very thick (Fig. 23), but appears to be slightly elastic because of the bulging shown in the gravid proglottides due to the expanding uterus. No pre-formed or spontaneous uterine pore could be seen, so it is likely that the gravid proglottides become detached from the strobilus as they are tending to do in our specimen.

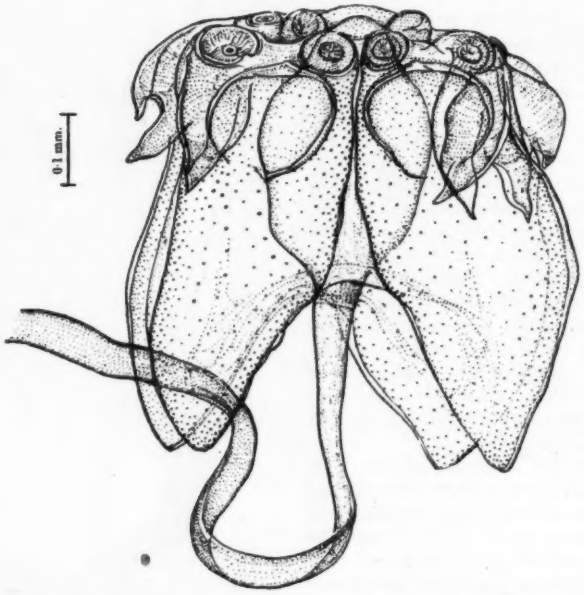


Fig. 20. *D. paciferum* n.sp. Whole mount of scolex of gravid strobilus.

It is not possible to make out the presence of bulbous vesicles similar to those of *D. planum* behind the bothria, nor could the excretory vessels be traced in the anterior region of the strobilus.

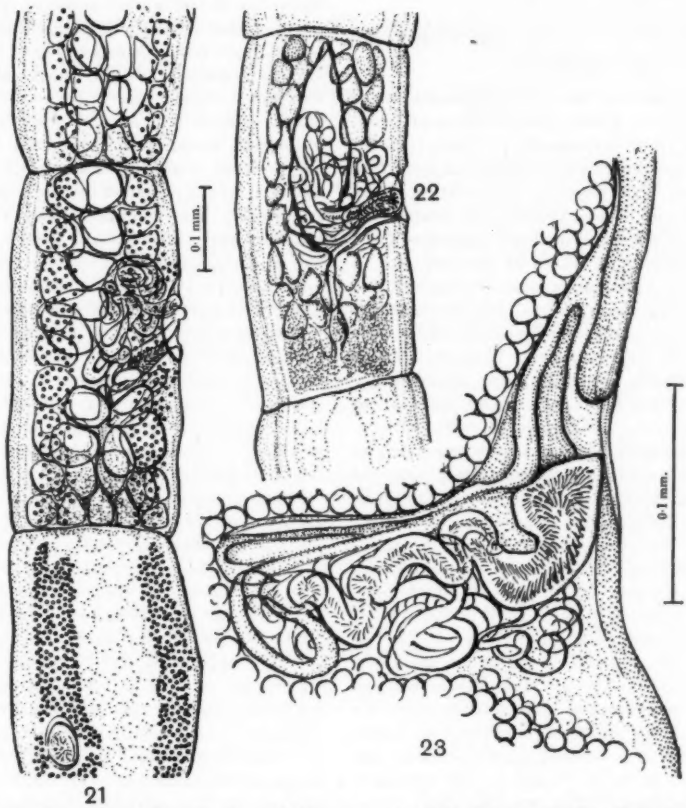
The shorter specimen retains the larval tail which bears a minute excretory pore at its tip; the excretory vessels do not appear to open by special slits at the posterior ends of each of the proglottides as in other species, and in the middle region it was thought that a transverse excretory vessel could be seen (Fig. 22). The genital pores are irregularly alternate and occur slightly in front of the middle of the proglottis.

The very darkly pigmented vitellaria are a

It is interesting to note that the maximum development of the vitellaria, in both strobili (though to a different extent) occurs in proglottides at the end of the 'male phase' and the beginning of the 'female phase', as previously noticed in *D. planum* and *D. keilini*. In Fig. 21 (taken from proglottides *a*, *b*, *c* of Fig. 19) in which the vitellaria are accumulating as increasingly dense and wide lateral bands, the testes are turgid and ripe; whereas in Fig. 22 (taken from proglottis *d* of Fig. 19—three beyond the rather feeble maximum development of vitellaria in this strobilus) the testes are shrunken, and some are spent, appearing sparsely granular or hollow. In Fig. 22 the ovary is conspicuous, but the inflated

uterus is as yet quite empty: in front of it in the figure can be seen the distended coils of the vagina and vas deferens, and it is noteworthy that the vagina has a tendency to grow in an anterior direction as is characteristic of *D. septaria* and *D. keilini* (to a far greater extent), and of *D. planum* to only a slight extent.

proximal portion is lined with very strong spines; and the longer distal portion, with relatively thick walls has spines of about half the length. The opening of the vagina is curious in that it appears to be guarded by a forwardly directed flange, causing a 'hair-pin bend' to occur inside the entrance. Thence its course is directed straight inwards, parallel to the



Figs. 21–23. *D. paciferum* n.sp. Fig. 21. Proglottides *a*, *b*, *c* (see Fig. 19) showing the fully developed testes, in two layers, overlain by vitellaria (shown diagramatically here and elsewhere by coarse stippling), and the developing ovary at the base of the proglottides *a* and *b*. Fig. 22. Proglottis (*d*, see Fig. 19) at the end of the male phase, showing some spent testes, distended genital ducts convoluting in the forward region, the fully developed ovary and the empty uterus occupying the median field in the upper half of the proglottis. Fig. 23. Terminal region of genital ducts and portion of uterus of gravid proglottis (*e*, see Fig. 19). Note the 'baffle-wall' at the entrance to the vagina and the introverted cirrus with its two sets of spines.

The ripe testes are relatively very large and they assume various shapes due to mutual pressure, but their greatest diameter is usually between 48–55 μ, and there are about 40 in each proglottis. The cirrus is voluminous and enclosed in a very thin cirrus-sac (not shown in Fig. 23, which is taken from the poral region of proglottis *e* of Fig. 19), its short wide cirrus-sac, and when space allows it, sends its convolutions forwards accompanied by the coils of the vas deferens; but in gravid proglottides (Fig. 23) the pressure of the expanding uterus compresses both the coiling ducts into a conical region round the genital pore.

The uterus develops in two main lobes: in front

and behind the genital pore, and each main pouch has several subsidiary pouches. In even the most distended proglottis there are paler channels seen in the uterus which appear to be branching ducts supplying it. None of this material was sectioned, so that little detail could be made out in the thicker regions. The eggs are spherical and measure 17–18·5 μ in diameter.

THE SYSTEMATICS OF *DINOBOTHRIUM* VAN BENEDEN

Woodland's studies on the Tetraphyllidea have enabled him amply to justify the retention of *Dinobothrium* van Beneden, in the family Phyllobothriidae as a distinct genus from *Phyllobothrium* van Beneden, 1849. The burden of his paper on the revision of the classification of this order (1927*b*) is to emphasize the importance of the structural relationships of the proglottis as family characters at the expense of those of the scolex. Thus, he redefines Tetraphyllidea to exclude the two other orders, Pseudophyllidea and Cyclophyllidea, and in the first he includes only three families: Phyllobothriidae, Tetrarhynchidae and Proteocephalidae. Since the status of the family Onchobothriidae depends only on the presence of hooks on the scolex, he merges it with Phyllobothriidae *auctorum* which he redefines; should it be felt necessary, however, to create a 'purely artificial separation' he invites the revival of 'the original subfamilies of Phyllacanthinae and Phyllobothrinae (Carus, 1885)'. In parentheses, it may be mentioned that the first name would not be permissable, since there is no genus to support it; 'Onchobothriinae' could take its place. In Woodland's revised sense, the essential characters of Phyllobothriidae are: the presence of phyllidea unassociated with proboscides, a single zone of longitudinal muscle bundles more or less co-extensive with the subcuticula, an ovary which in transverse section is X-shaped, and with a uterine duct dorsal to the vagina which is, in turn, dorsal to an extensive uterine sac (Woodland, 1927*b*, pp. 532, 533).

The genus *Dinobothrium* is remarkable in several respects, and though we have not yet learnt what is artificial and what is natural in the generic sense, in this family, it is certainly the characters of the scolex which hold these four species together. In no other phyllobothriids are there developed the flexed bifid lobes at the outer ends of the bothridial crest: they have a curious constancy of form in all four species, and were they more heavily cuticularized, they would be taken for four 'broad hooks'. From this point of view the genus comes near *Pedibothrium* Linton, 1908 (except that there are two bifid hooks in this genus which lie on each bothrium), and *Ceratobothrium* Monticelli, 1892—both formerly placed in Onchobothriidae. Incidentally, Joyeux & Baer (1936, p. 49) consider the latter to come in Phyllobothriidae; though Yamaguti (1934, pp. 69–71), who has redescribed *C. xanthocephalum*, places it in Onchobothriidae, yet he admits that the 'hooks' are 'horn-like appendages', i.e. not fully cuticularized. His description of this species (1934, fig. 112) shows that the mature proglottis has much in common with that of *Dinobothrium septaria*—notably the extension of the convolutions of the vagina to near the anterior end of the elongated proglottis—the cirrus, however, is less developed and lacks spines, and the vitellaria are far more restricted laterally. Proglottis characters of *D. keilini* are even more closely paralleled in *Pelichnibothrium speciosum* Monticelli 1889, as described for the first time in the mature state by Yamaguti (1934, pp. 71–3, figs. 113–16) and placed by him in a new monotypic subfamily. The vagina passes forwards to the anterior end of the uterus before descending, and the convolutions of the vas deferens are developed anteriorly to it; the cirrus is lined with spines, and a sheet of vitelline follicles covers both sides of the proglottis except for the median field. The entire bothria have one small sucker at their apex, also as in *Dinobothrium keilini*; but here the resemblance ceases, for the ovary is composed of two compact lobes, there is no apical ridge on the scolex ending in pairs of 'reflexed bifid lobes', and there is a terminal sucker like those above the bothria.

On the other hand, according to the primary family criteria laid down by Woodland (1927*b*, pp. 532–3), *Dinobothrium* spp. show some relationship with Tetrarhynchidae in one of his 'deep-seated' characters, namely the tendency of the vitellaria, at their maximum development, to encircle the proglottis; albeit shrinking back to the typical marginal bands by the time the female phase gets under way. Woodland affirms, without giving his rationale, that this peripheral continuity of the vitellaria 'in the young proglottides of some species (of Phyllobothriidae) is probably reminiscent of an ancestral condition' (1927*b*, p. 532). However this may be, the condition is shown to a diminishing extent in the series: *D. paciferum*, *D. planum*, *D. keilini*, and *D. septaria* as described above.

Some time ago, Baylis (1926, pp. 171–2 (the paper having being read before the Linnean Society of London on 20 November 1924)) drew attention to the striking similarity in the scolex of *Tetrabothrius* spp. and that of *Dinobothrium* spp. He was dealing with the different forms exhibited by the scolex of *Tetrabothrius affinis* (Lönnberg, in Jägerskiold, 1891) from balaenopterid whales: these have the bothria-pairs connected above by a similar flat crest, ending in 'auriculate appendages' or 'epaulettes', and in addition the similarity is increased by a bilobed portion overhanging the junction of the bothria in the middle line (analogous to the pseudo-suckers of

Dinobothrium planum). Baylis concluded by suggesting that *Dinobothrium* van Beneden might be regarded as belonging to Tetrabothriidae; he was, however, handicapped in not having any comparative material at hand. It was not until October 1925 that Woodland (1927*a*) recovered specimens of *D. septaria* from a *Lamna cornubica* landed at Plymouth, and by the study of which he was able to show the essentially a persistant larval form: it is the first organized part of the animal to be laid down, and with but slight modification, it remains as the *sine qua non* for the maintenance of the parasite. The structure of the scolex, therefore, cannot be without significance in the study of phylogenetic relationships, so it is still worth considering that part of Baylis's conclusion (1926, p. 172) in which he says

The characters of the species of Dinobothrium *van Beneden*

	D. septaria	*D. keilini*	*D. planum*	*D. paciferum*
Host	*Lamna cornubica* and *Carcharodon carcharias*	*Carcharinus glaucus*	*Cetorhinus maximus*	*Cetorhinus maximus*
Scolex	5–7 × 7–9 mm.	8·3 × 10 mm.	6·7–8·0 × 8–10	0·65 × 0·65 mm.
Apical 'suckers'	4 pseudo-suckers	4 true suckers	4 arched pseudo-suckers and 4 true suckers	8 true suckers
Crest	Prongs in mid-line from shelving roof	Passes smoothly round bothria	Shelving roof	Inconspicuous fold
Ends of bothria	Free for ⅓–½ their length. End in double point from furrow	Narrow margin only free. Rounded angle	Free for ⅕–¼ their length. Obtuse angle	Free for ½ their length. Pendant ends with acute angle
Neck	None. Ventral furrow strongly marked	Short. Ventral furrow slight	None. Ventral furrow marked	Very long. No furrow
Proglottides	Narrow, square, to long (3·5 × 1·5 mm.)	Narrow, barrel-shaped, to slightly elongated (3·7 × 2·1 mm.)	Narrow (1·0 × 3·7 mm.)	Cylindrical elongated (0·7 × 0·45 mm.)
Vagina	Coils to anterior end. (?) 2 sphincters	Coils to anterior end. 2 sphincters	Only slightly directed anteriorly. No sphincters	Anterior tendency in early coiling. No sphincters
Vitellaria	Semicircle outside excretory vessels; less than ½ across proglottis	Wide bands outside excretory vessels; more than ½ across proglottis	⅔ across ventral surface at first, then narrow lateral bands	All round proglottis at first, then narrow lateral bands
Follicles	40 × 26 μ	21–33 × 75–58 (100) μ	30 × 50 μ	Ill-defined
Testes	51 × 47 μ	92–125 × 66–87 μ	80 × 50 μ (*c.* 300 per segment)	48–55 × 18–21 μ (40 per segment)
Eggs	25·6 μ diam.	27·5 × 22·5, 32 × 19 μ	17·4 μ diam.	17–18·5 μ diam.
Strobilus length to gravid segment	100–300 mm.	?	378–397 mm.	11 mm.
Total length of strobilus	110–300 mm.	?	500–600 up to 825 mm.	17·2 mm.

fundamental difference in internal anatomy between these two genera. The subsequent discovery of other species of *Tetrabothrius*, notably from birds in America by Linton (1927, figs. 6–39), has further confirmed the close analogy between the scolices of the two genera, and in spite of the accepted systematic importance of the general anatomical structure of the proglottides, it is irrational to ignore so important an organ as the scolex. The scolex of a cestode is

'it is not impossible that through such forms as *Tetrabothrius* and *Dinobothrium* the groups Cyclophyllidea and Tetraphyllidea grade into each other, and are perhaps also connected with the Pseudophyllidea'.

Woodland objected to Southwell's inclusion of this genus in *Phyllobothrium* van Beneden on the grounds of the bothria of both groups possessing 'accessory suckers'; and apart from demonstrating

that the type species lacks a functional sucker, Woodland thought that the scolex characters provided a good basis for the genus *Dinobothrium*. His definition (1927*a*, p. 241) being: those phyllobothriids with 'a one-loculed, crested bothridium'; but as this is scarcely adequate for the exclusion of certain *Tetrabothrius*-like species of uncertain family status, a fuller diagnosis is given below.

It may be mentioned that there is a feature of the bothria which is so far infallible in distinguishing the similar scolices of these two genera: in *Tetrabothrius* spp. each bothrium is provided with a strong marginal sphincter, which accounts for the cup-like appearance in some contracted specimens. Up till now this has not been seen in any *Dinobothrium* species, though *D. keilini*, the most muscular of them, has a marginal flange of its bothria free from the scolex, but in spite of appearances in Fig. 12, this is not specially muscular. The more contracted specimens of *D. planum* show that the bothria tend to fold longitudinally, like the in-folding edges of a leaf, and are not in the least cup-shaped as in many *Tetrabothrius* species; this tetrabothriid feature is shown, incidentally by *Diplobothrium simile* van Beneden, 1889.

Generic diagnosis of Dinobothrium *van Beneden*

Phyllobothriidae with a squarish scolex without hooks, and in which the bothria have entire margins, without a sphincter; they are more or less adpressed to the axis of the scolex, and joined anteriorly to a transverse crest, at the ends of which are pairs of reflexed bifid lobes with broad points. On the crest above each bothrium is one or more suckers, pseudosuckers, or both; a central rhynchus is not developed. The vitellaria tend to spread round the proglottis in the early female phase of its development, but later shrink back to narrower marginal bands. The vagina and vas deferens are voluminous and act as sperm reservoirs; the cirrus is lined with spines and the eggs are non-filamented.

Genotype: *Dinobothrium septaria* van Beneden, 1889.

A NOTE ON A SPECIES OF *MONORYGMA* FROM THE ELECTRIC RAY

The single immature strobilus from *Torpedo nobiliana* (see p. 74) was showing signs of segmentation, but only the anlages of organs were present in the nascent proglottides—that of the genital pore being about mid-marginal. The total length of the strobilus is 21 mm. more than half of which is 'neck'; the first segments are brick-like in proportions, increasing in length till the penultimate is nearly square, the last is the larval tail with a prominent excretory pore at its tip.

The squarish scolex (0·665 mm. long and wide) is distinctive and cannot be identified with any species so far described or figured; nevertheless, because of the absence of proglottis characters it is not proposed to name it, but to assign it provisionally to the genus *Monorygma* Diesing, 1863. (The assignation is in accordance with the keys and definitions given by Meggitt, 1924, pp. 136–7, 140, and Linton, 1924, pp. 15, 16–20.) Fig. 24 shows the scolex with the scar left by the detachment of one of the bothria, which is shown separately.

The four bothria are boat-shaped and stand out from the scolex in a cruciform arrangement: each is held by a thin wing composed of transverse fibres. This wing is only about two-thirds the length of the bothrium and is attached anteriorly just below the apical sucker, leaving a faint line as shown (this is not a sphincter nor, apparently, an incipient partition as seen in young *Calliobothrium* spp.). There is another membrane of transverse fibres, attached to the inner half of the circumference of the suckers on the one hand, and on the other, passing towards the centre of the scolex at its apex—where there is a slight indication of an apical organ. By the contraction of the apical membranes the bothria are splayed outwards, and their free ends can describe a considerable arc; but they can be pulled back rapidly to the scolex by the compensating action of the contraction of the wing-like membranes below the sucker.

The suckers are complex, their apertures having different shapes as the focus is deepened. Superficially there are two lips tending to close the cavity from the axial side, slightly lower there is a crescentric thickening of the outer arc of the sucker, and this is continued below the two broad lips. At a slightly lower level again, there is a muscular rim surrounding a rounded-triangular aperture to the inner cavity. The detached bothrium (Fig. 25) shows these details; the edges of the bothrium itself are continuous round the sucker, they are slightly inrolled, but are not supplied with a sphincter or any other special musculature. An excretory vessel of one side can be seen entering the scolex in the figure; but no other structural details could be made out.

Monorygma (*Anthobothrium*) *perfectum* (van Beneden, 1858) Diesing 1863, has been redescribed by Linton (1924, figs. 18–31) from *Somniosus brevipinna*, and the scolex agrees fairly well with the original description of worms from *Scymnus glacialis* (see Southwell, 1925, fig. 93). In some ways this species comes near to the present one, notably in the proportions of the suckers to the bothria, but not in the shape of the latter—which is pyriform and thus deeply hollowed, and with a wide neck, so that they are certainly not conspecific. Though *Monorygma perfectum* has been recorded from several sharks, it has never been found in a ray. Incidentally, Southwell (1925) makes *Orygmatobothrium forte* Linton 1924 (figs. 8–10) conspecific—it does not

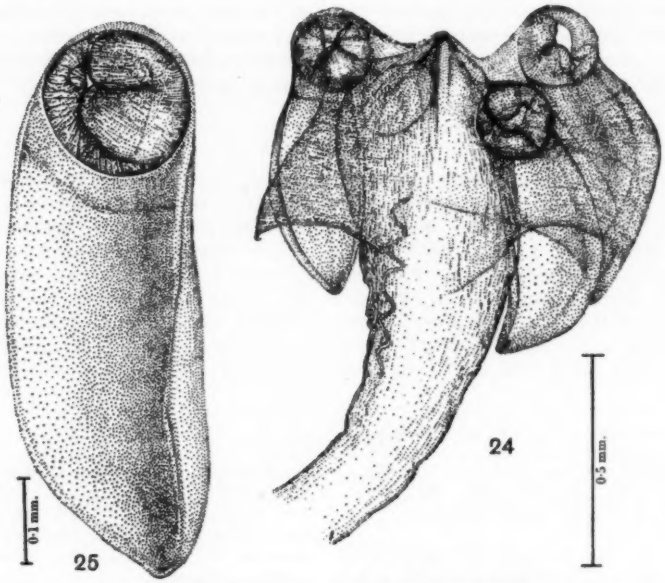
appear even to be congeneric, owing to the two concentric sphincters of the bothria. On the other hand, insufficient evidence is given by its author to show that *Phyllobothrium magnum* Hart, 1936 (pp. 489–92, figs. 1–4) is not identical with *M. perfectum* (van Beneden) and of Linton, 1924.

The original descriptions and figures of *Orygmatobothrium angustum* Linton, 1889 (pp. 468–9, pl. iii, figs. 1–3) from *Carcharias obscurus* show a scolex very near to the present species, but though the size and shape of the bothria are in agreement, the size of the suckers appears to be considerably smaller. Yoshida (1917, pp. 563–6, figs. 1–5) gives a redescription, but again no mention is made of the size of the suckers. This author transfers the species to *Crossobothrium* Linton 1889, the reason being obscure; but as *Orygmatobothrium* Diesing 1863 was created for those phyllobothriids with an additional central sucker on the bothrium, it would seem that Linton erred originally, and that it should be *Monorygma angustum* (Linton).

Of the phyllobothriids characteristic of *Torpedo* spp., the nearest to the present species is *Calyptrobothrium minus* Linton 1907, which is of nearly the same size and proportions. The fundamental difference however, is the shape of the bothridial sucker, which in *Calyptrobothrium* Montiecelli is always horse-shoe-shaped, with the posterior side open to the slipper-shaped bothrium. On the available evidence, therefore, it appears that the present species is possibly new, and it may represent a distinct genus having relationships in regard to the scolex, with *Monorygma* and *Calyptrobothrium*.

SUMMARY

A new species of the giant phyllobothriid genus *Dinobothrium* (*D. keilini* n.sp.) is described from the blue shark (*Carcharinus glaucus*); it is compared with *D. planum* Linton, found for the first time from British waters, in the basking shark (*Cetorhinus maximus*), and the structure of both is discussed in relation to that of the genotype, *D. septaria* van Beneden. A fourth species, *D. paciferum* n.sp., also from the basking shark, is described, and though only a tenth the size of the other species, it shares most of their diagnostic scolex characters. It has eight true suckers above the four pendent bothria: in *D. planum* there are four true suckers and four pseudo-suckers, while in *D. keilini* there are only four true suckers, and *D. septaria* is said to have four



Figs. 24, 25. *Monorygma* sp., from *Torpedo nobiliana*.

Fig. 24. Scolex from the immature strobilus (whole mount with one bothrium removed, but leaving its supporting wing). Fig. 25. The detached bothrium from scolex in Fig. 24, particularly to show the complicated structure of the sucker, and the line of attachment of the upper margin of the supporting wing—virtually the pivotal line taking the main strains when the bothrium is swung outwards and inwards by the contraction of the upper and lower supporting membranes, respectively.

pseudo-suckers alone. The nomenclature of the older species is revised, and the genus redefined.

Attention is directed to the adaptations in these large cestodes which facilitate interchange with the substratum: the development of lateral furrows through which the main excretory canals open to the exterior. There is also a ventral furrow, which in the nascent segments is an open cleft: this accommodates the growing uterus and permits a protracted gravid phase of the proglottides *in situ*. The effect is of five longitudinal hinges, which allow of considerable lateral expansion of the thick-walled strobilus. The smaller *D. paciferum* lacks these modifications, and the cylindrical segments are apparently detached in the gravid state. An explanation is offered for the conflicting accounts of the lateral extent of the vitellaria in this genus: there is a lateral contraction of the ventral vitelline reticulum as the ovarian phase waxes. In *D. paciferum* the vitellaria completely encircle the proglottis at the inception of the ovarian phase.

Notes are given on the selachian hosts examined, their food and the other parasites present. The relationships of *Dinobothrium* to other phyllobothriid genera and to *Tetrabothrius* are discussed.

In one electric ray, *Torpedo nobiliana*, a single immature strobilus of another kind of phyllobothriid was found; it is probably a new species of *Monorygma*, though it has slight resemblances to *Calyptrobothrium*—a genus characteristic of electric rays. The boat-shaped bothria are held at an angle from the scolex by contractile buttress-like wings, and at their proximal end there is a relatively large and complex sucker. Affinities with related genera are discussed and the nomenclature of *Monorygma angusta* (Linton) is emended.

ADDENDUM

Since arriving in China the following paper by Dr S. Tseng, on the cestodes of fishes, has come to my notice (*J. Sci. Nat. Univ. Shantung, Tsingtao, China* (1933), vol. 1, pt. 2, pp. 327–50, 27 figs.—in Chinese). He describes and figures (p. 330, Figs. 6–7) an immature strobilus of *Dinobothrium septario* van Ben. (*sic*) from the intestine of *Cetorhinus maximus* caught off Shantung in the summer of 1932. The shark was 14 ft. long and weighed some 600 kg. The short description and measurements of the scolex are in agreement with those of *Dinobothrium planum* Lint., and the figures amply confirm this identification. The strobilus was only 43 mm. long, and apart from the strong ventral groove no internal structures could be made out. The finding is of considerable interest in that it also indicates the cosmopolitan distribution of the intermediate host or hosts.

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CULTIVATION OF *TRYPANOSOMA CRUZI* IN TISSUE CULTURES: A FOUR-YEAR STUDY*

By H. MEYER and M. XAVIER DE OLIVEIRA

(With Plates II and III)

In 1941 we began the cultivation of *Trypanosoma cruzi* in tissue cultures. In a paper published in 1942 we showed that *T. cruzi* is easily cultivated in tissue cultures using the hanging-drop method of Carrel, a method which has also been previously used by Kofoid, Wood & McNeil (1935). In our cultures it was possible to observe, *in vivo*, the parasite during all phases of its intracellular cycle, the transformation of the flagellate form into the crithidia and leishmania form, the multiplication of the parasite in the crithidia and leishmania form by binary division, and finally the transformation back to the flagellate form, thus confirming the classic knowledge of the evolution of *T. cruzi*. We studied further the behaviour of the cells containing parasites, the process of cellular disintegration, and saw that the trypanosome thrived in every cell available to it, and in 8 months of cultivation lost none of its virulence.

The ease with which the trypanosome was cultivated in tissue cultures suggested a series of morphological studies not only of the parasite but also of the parasite-containing cells, which were carried out in the following years, and which are summarized in this paper as a résumé of about 4 years of observation of *T. cruzi* in tissue cultures.

MATERIAL AND TECHNIQUE

The technique used was always the classic technique of Carrel of hanging-drop cultures, slightly modified in order to obtain extremely thin cultures. Thus, we did not transfer the cultures three times a week, but only every fifth day, washing them every second day with Ringer's solution and diluted embryonic juice. We used exclusively tissues of chick embryos. In order to infect the tissue cultures with *T. cruzi* we placed a drop of a blood agar culture on top of the explant. If a tissue culture had already many parasites, we added at the next transfer a small piece of fresh tissue, which the trypanosomes invaded and infected. Thus it was possible to cultivate the trypanosome for more than 3 years in chick embryo cells.

* This work was done in the Institute of Biophysics of the University of Brazil under the direction of Prof. C. Chagas, to whom we express our gratitude.

We studied the parasite-containing cells, *in vivo*, under the microscope, at 38–39° C., and, when necessary, we fixed the culture *in toto* with Bouin or Zenker fluids and stained with ferric haematoxylin.

RESULTS AND DISCUSSION

Inoculation in animals. At various intervals we inoculated a culture containing many parasites subcutaneously or intraperitoneally in a white mouse, and it was seen that after 3 years of cultivation in chick embryo cells the trypanosome did not lose its capacity to infect mice, which always showed parasites in their blood about 12 days after inoculation.

It is well known that the chick is refractory to the inoculation of *T. cruzi*. As it was so easy to cultivate the trypanosome in chicken cells we tried many times to infect newborn chicks with blood agar cultures or infected tissue cultures, but we never succeeded, even with trypanosomes that had been cultivated for 2 years and 3 months exclusively in chicken cells, to produce infection in chicks.

Cycle. T. cruzi inoculated into animals penetrates into the cells, assumes the leishmania form, and after 4 days this form changes back into trypanosomes. Kofoid *et al.* also observed a 5 days' cycle in their cultures. Examination of our thin cultures under high magnification revealed new trypanosomes after only 4 days, or, in some cases, 3 days after inoculation. This 4–5 days' cycle takes place very regularly in tissue cultures. We thought first that this regularity might be explained by the fact that the parasite must undergo a certain number of divisions in the leishmania form in order to reach the flagellate form, but we soon noticed that sometimes there was a large number of trypanosomes formed at the end of a cycle and at other times very few. Accordingly, we studied the relation that exists between the host cell and the proliferation of the parasites in the cell's protoplasma (Meyer & Xavier de Oliveira, 1944), and we saw that the number of leishmania or trypanosomes, produced at the end of a cycle, varied greatly according to the size of a cell. Small cells, for example fibrocytes, had about 50–70–100 trypanosomes at the end of the cycle, and large cells,

such as nerve cells, had up to 300 (cp. Pl. II, figs. 3–7). Evidently it is the host, that is to say the medium in which the parasite finds itself, that determines the number of divisions of the parasite.

We saw that the cycle of the trypanosome is invariably limited to one cell unit, and we observed that the adult form is never restricted to any one part of the cell, but rather the parasite in the leishmania form continues to divide in this form until the cell is full (Pl. II, figs. 1–3). The transformation into the flagellate form begins only at this moment. During the first days of infection, when there are only a few leishmania forms, and when it is still easy to count them, it is seen that the parasite undergoes binary fission about every 12 hr. With this rhythm a medium-sized cell at the end of the third day, or at the beginning of the fourth, contains about 120 parasites, which completely fill it. In the majority of the cells studied, the transformation to the flagellate form begins at this point. Very small cells become filled earlier, and in these the adult forms emerge earlier, on the fourth or third day. The transformation into the final form, passing through the crithidia form, begins almost simultaneously in all leishmania forms (Pl. II, fig. 4), and is complete within 24 hr. The leishmania form, which during the first 3 days shows only a very slight rotary movement, begins to put out a small flagellum, the round shape becomes more elongated, and the movement progressively more rapid. In many cells observed under the microscope, at 38–39° C., we saw that the transformation is complete and the trypanosomes leave the cells from 12 to 24 hr. after the first movement of the parasites has been noticed.

We attempted to prolong or to shorten the 4–5 days' cycle experimentally, and it was found that in cultures which are maintained at room temperature (Meyer & Xavier de Oliveira, 1943) the cycle is delayed, and also in cultures infrequently transferred, where the rate of cell proliferation is decreased, there are cells in which the number of parasites do not increase further and the transformation into the flagellate form occurs only after 8 days. It appears that there is a relation between the state of metabolism of the cell protoplasm and the parasite contained in it. Further experiments must be carried out in order to clarify this relationship. We have not yet succeeded in shortening the cycle.

Dimorphism. Another point that should be investigated more thoroughly is the question of dimorphism of the adult form of the trypanosome. The prevailing opinion is that the thin form of the trypanosome represents a younger form of the parasite, because it appears first in the blood after inoculation. In tissue cultures we are able to observe the final phase of the intracellular development of the parasite and the exit of the trypanosomes recently formed in the cells. It was seen that the trypanosome immediately after the crithidia phase is broad and short (Pl. II, fig. 4). There are cells which poorly resist the active intracellular movement of the trypanosomes or the extracellular influences, such as lysing by the medium, and rupture before all the parasites within have completed their evolution, for example, when some of the parasites are still in the crithidia form. In this manner many immature forms leave the cells, and with them the first adult forms, which are broad and short, and which usually spend many hours moving continuously about the site of their exit, never progressing very far from the destroyed cell (Pl. II, figs. 8, 9). Other cells, due to a more resistant surface, are able to resist longer their external environment or the great internal activity of the trypanosomes. It is in these cells that we find the thin forms of the parasite which, after being freed, immediately leave the place of exit (Pl. II, fig. 7, Pl. III, fig. 10; cp. Pl. II, figs. 4, 7, 8 and 9 and Pl. III, fig. 10, all taken with the same magnification). The fact that these thin forms appear first in the circulating blood is probably due to their greater mobility. It has not yet been possible to measure and to follow one large living trypanosome closely to see if it changes into the thin form, because the great number of trypanosomes leaving the cell, coupled with their great activity, makes it impossible to recognize the same trypanosome after a few hours.

We never observed mating between the broad and the thin trypanosomes. Very large forms, which were twice the normal size, were followed for several hours. They invariably showed degenerative changes or they did not change at all. In stained preparations these gigantic forms were often seen to contain two nuclei and two blepharoplasts, two nuclei and one blepharoplast, or, vice versa, two blepharoplasts and only one nucleus (Pl. III, fig. 11). The fact that these forms were never seen to become transformed or to divide led us to believe that we were dealing with abnormal forms.

Division of the adult trypanosome. As to the binary division of the adult trypanosome (Meyer, 1944) that was observed in exceptional cases, the following may be said: In four cases very large trypanosomes were seen leaving the destroyed cell, and immediately thereafter divided into two. It is possible that in these four cases we may have been dealing with two trypanosomes adhering to one another by means of protoplasm that remained from the destroyed cell. *In vivo*, it was not possible to see this protoplasmic membrane, and the process of 'division', or better separation, was always very rapid (Pl. III, fig. 12).

At the time we published a previous note about these phenomena we considered certain biflagellate and bifurcated forms (Pl. III, fig. 13) to be, perhaps, forms in the process of division. However, after more numerous and more prolonged observations, we now consider these forms also to be anomalous,

monstrosities, incomplete divisions of crithidia forms, because complete division was never seen, even after 24 hr. of observation. Sometimes they degenerated rapidly.

Behaviour of the parasite-containing cells. Concerning the parasite-containing cells we found that during the first days of infection they show no signs of degeneration. The migration, pulsation and proliferation continue, even when a great part of their protoplasm is already filled with the leishmania form. The mitotic process was studied in greater detail (Meyer & Xavier de Oliveira, 1942), and was seen to occur in the normal time. At times the separation of the two daughter cells was retarded because a group of parasites was at the site of the separation. The distribution of the parasites in the cytoplasm during the division was irregular. At times there was an equal distribution, at other times it was unequal, and sometimes all of the parasites remained in only one of the daughter cells (Pl. III, figs. 14, 15). When the cells are completely filled signs of degeneration are seen, especially in the nucleus, which becomes irregular and opaque. The cytoplasm becomes vacuolated, but, in general, this is difficult to observe because it is completely filled with parasites. As has already been noted there are cells with delicate surfaces which are vulnerable to the internal movement of the trypanosome or to extracellular influences, and such cells rupture before the cycle is complete, even when the parasites are still in the leishmania form. Macrophages, for example, which have the most delicate surface of all cells, rupture easily in the zones of liquefaction, whereas other cells, such as nerve or muscle cells, remain for a long time with the trypanosomes undergoing intense movement, appearing under the compound microscope like highly agitated protoplasmic sacs which rupture suddenly and the freed trypanosomes spread out rapidly from the point of exit. This final act of rupturing, and of the exit of the trypanosomes, is different in different cells. Sometimes the cells retract their extensions, become globular and appear in the microscopic field like a cyst with great internal movement, and then finally rupture (Pl. III, fig. 16). There are others, however, that do not retract, and the typanosomes move out slowly, a few at a time. At other times, cells with very viscous protoplasm were seen, from which it is difficult for the trypanosomes to liberate themselves (Pl. III, fig. 12). In still others the cell expels small protoplasmic sacs, containing very active trypanosomes, which gyrate about the field for some time and then, they too, finally rupture.

In tissue cultures the trypanosome always completes its cycle in one cell unit. The leishmania form never passes from one cell to another. The neighbouring cells of a cell, destroyed by parasitism, remain intact (Pl. II, figs. 6, 7). Several times adjacent cells were observed which contained parasites in different phases of their evolution. This proves that each cell maintains its autonomy during the infection. Even in the so-called cell syncytiums, such as muscle fibres, where the cycle is limited to only one cell (Pl. III, fig. 17), the parasite did not fill the entire fibre.

SUMMARY

Trypanosoma cruzi is easily cultivated in tissue cultures of chick embryos, but it was not possible to infect chicks with trypanosomes cultivated for more than 2 years in chicken cells.

The trypanosome cycle takes place in one cell unit.

The number of trypanosomes formed in one cycle depends on the volume of the host cell.

The transformation of the leishmania form into the flagellate form begins when the cell is full of parasites, and occurs in 12–24 hr.

The intracellular trypanosome, immediately after the crithidia form, is broad and short, the thin form probably being a later phase of the evolution of the parasite.

Division of the adult trypanosome, or mating between the thin and broad forms, was never seen.

At the beginning of the infection the cells withstand the parasites and continue to proliferate, but finally they are destroyed.

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EXPLANATION OF PLATES II AND III

(In all cases except the preparation shown in fig. 14, the material was fixed with Zenker's fluid and stained with Heidenhain's iron haematoxylin

PLATE II

Figs. 1–3. Monocytes from blood of adult chick. Parasite in all phases of its cycle. (1) Only three leishmania forms, the big one in division. (2) Cell already full. (3) Parasite in flagellate form, final phase of the intracellular evolution.

Fig. 4. Fibrocyte from heart muscle. Parasites in process of transformation to the flagellate form.

Fig. 5. Small histiocyte from spleen, small number of parasites in adult form.

Fig. 6. Epithelium from amniotic sac. Parasites in leishmania form, some of them still undergoing binary fission.

Fig. 7. Epithelium from amniotic sac. Parasites in adult form, final phase of intracellular evolution.

Fig. 8. Cell from spleen culture ruptured before all the parasites have completed their evolution. Broad and short forms.

Fig. 9. Cell from spleen culture, ruptured before all the parasites have completed their cycle. Broad and short forms.

PLATE III

Fig. 10. Heart muscle cell. All parasites in final phase (thin forms) of evolution.

Fig. 11. Abnormal form of trypanosome with 2 nuclei and protoplasmic adherence.

Fig. 12. Epithelium cell from liver. Parasites in final phase, leaving the cell. Two trypanosomes adherent to one another.

Fig. 13. Abnormal form of trypanosome with big blepharoplast, 1 nucleus and 2 flagella.

Fig. 14. Two fibrocytes from spleen, flattended, in mitotic division, containing parasites in leishmania form. Fixative Zenker, haematoxylin Ehrlich.

Fig. 15. Fibrocyte from heart muscle in mitotic division, containing parasites in leishmania form.

Fig. 16. Fibrocyte from spleen, retracted, parasites in adult form, leaving the cell.

Fig. 17. Muscle fibre from skeletal muscle. Only one cell of the fibre contains trypanosomes in the adult form.

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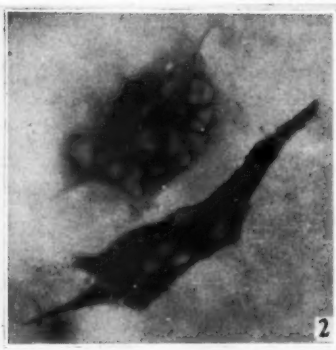
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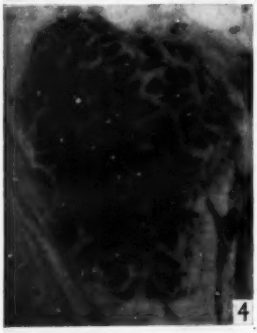
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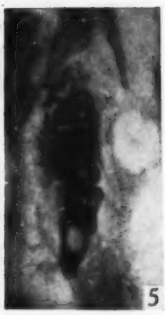
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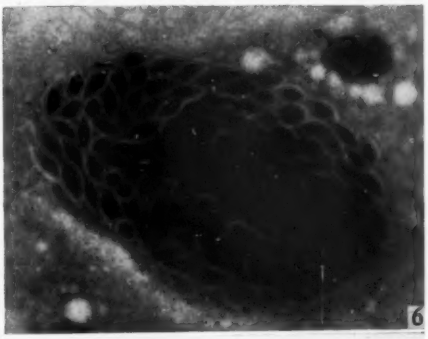
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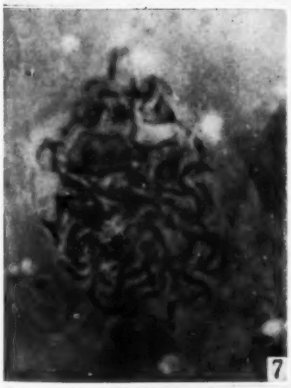
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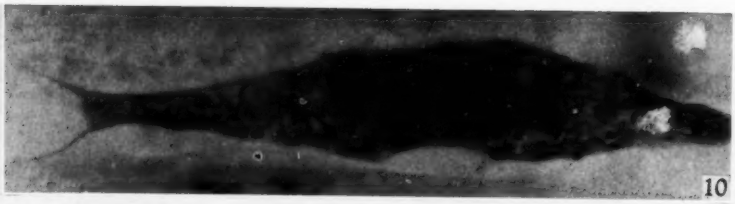
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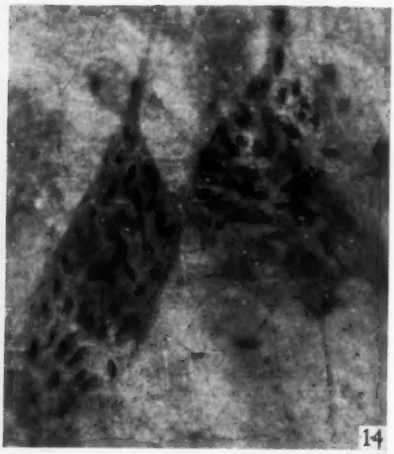
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A NOTE ON THE GENUS *HYALOMMA* KOCH IN PALESTINE

By S. ADLER and B. FELDMAN-MUEHSAM*

From the Department of Parasitology, The Hebrew University, Jerusalem

(With 8 Figures in the Text)

The diagnosis of species of *Hyalomma* has given rise to a good deal of controversy among the relatively few workers who have attempted the systematics of the genus. Koch (1847) recognized sixteen species. Neumann (1911) studied Koch's original material, together with additional extensive collections and recognized four certain and three doubtful species; all the specimens from domestic animals were included in one 'certain' species, *H. aegyptium*, and three doubtful ones. This opinion was generally accepted till Schulze undertook an extensive study of the genus. In a series of papers published between 1919 and 1935 Schulze and his collaborators affirmed the validity of Koch's species and created a considerable number of new ones. Schulze & Schlottke (1929) recognized twenty-seven species and twenty-nine subspecies, races and forms. In Schulze's classification fine differences in the colour of the legs and surface marking, particularly the grooves (which were subjected to an elaborate analysis and nomenclature), and the parma (a term introduced by Schulze for the central festoon of the male) are characters of prime diagnostic importance. Senevet (1922), in a study of the genus *Hyalomma* in Algeria, criticized Schulze's method because it involved a quadrinomenclature (e.g. *Hyalomma marginatum balcanicum brunnipes*), and depended on shades of colour so fine that differences in individual interpretation might readily occur. All workers agreed on the wide range of variations within each species, and for this reason Neumann had drastically reduced the number recorded from domestic animals. Schulze confidently maintained the validity of his species and sub-species 'trotz der großen Individualvariazionen bei *Hyalomma*'. Chodziedner (1924) accepted Schultze's method and explained the considerable variations within a single species on the supposition that *Hyalomma* is a relatively recent genus. Delpy (1936), after studying the genus, came to the conclusion that diagnosis of species is *almost impossible*. He suggested that the range of variations among laboratory bred offspring of individual females be studied; he himself had found considerable variations in the form of the subanal plate in a number of specimens of *H. dromedarii* derived from a single female. Later (1946) he again stressed the variations in laboratory bred specimens derived from individual females. Summarizing his observations on this aspect of the problem he remarked, 'Si l'examen est suffisament attentif, il devient impossible de trouver deux individus exactement semblable.' He mentions that Schulze, reporting on specimens which he (Delpy) had sent for determination, diagnosed some as *H. dromedarii* and others as *H. delpyi*, although all had been bred from a single female. Furthermore, he had bred specimens from a single female diagnosed by himself as *H. schulzei* on account of its large size, and because it was captured together with males of *H. schulzei*; the male offspring of this female were confidently diagnosed by himself as *H. dromedarii* (*H. schulzei*, ♂, is readily diagnosed because its stigmata are feminine in type).

It is, therefore, not surprising that identification is difficult and nomenclature confused; to quote but one example Sharif (1928), following Neumann, accepts *H. dromedarii* as a subspecies of *H. aegyptium* while both Schulze and Delpy consider them two distinct species.

The systematics of *Hyalomma* are not only of scientific interest but are also of prime economic importance, since Sergent, Donatien, Parrot & Lestostoguard (1928) have shown that at least one species, *H. mauritanicum*, transmits *Theileria dispar* in North Africa. We were faced with the problem of distinguishing species of *Hyalomma* as an unavoidable preliminary to determining the vector of *Theileria annulata* which is very common in Palestine. Like Senevet and Delpy we found Schulze's classification difficult, except in the case of *Hyalomma detritum* Schulze which is characterized by a yellow longitudinal band on the legs, a constant feature in the male.

As a first step (1946) we, like Delpy, studied the variations in the laboratory-bred progeny of individual females and found, in the case of *H. savignyi*, considerable variations in colour of the legs (from yellow to brown), in the shape and size of the parma (Fig. 1) (a character to which Schulze attaches great importance), in shape and size of

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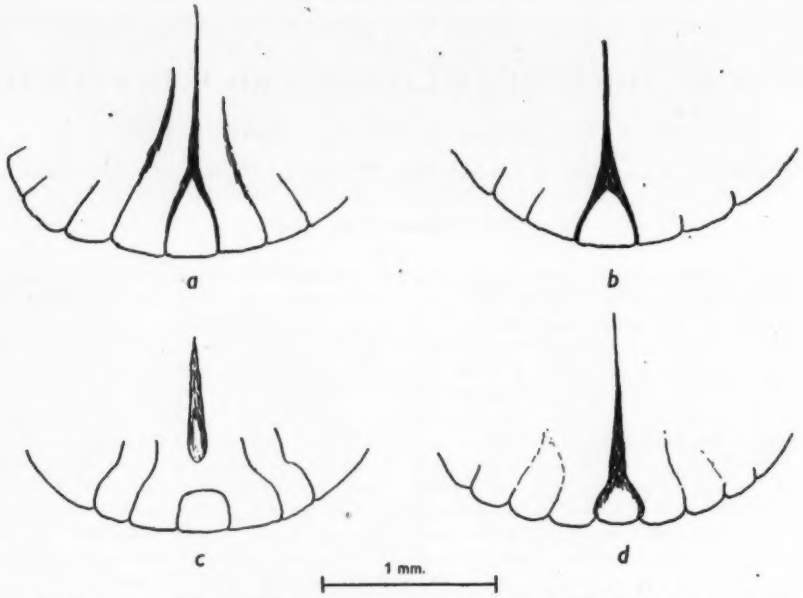

Fig. 1. Variation in parma of males of *H. savignyi* derived from a single female. Note in *c* presence of bridge above parma and absence of bridge in three other specimens.

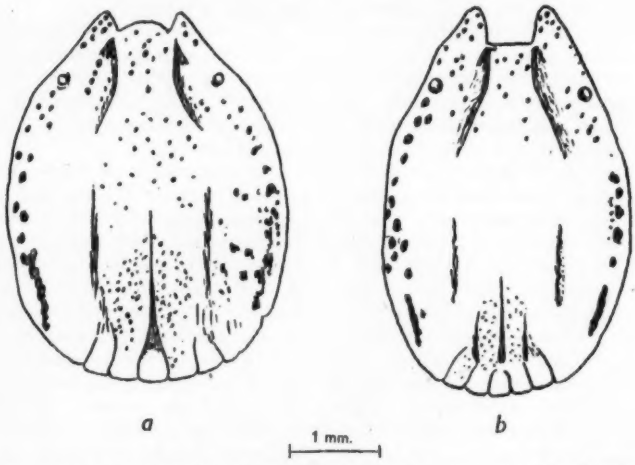

Fig. 2. *H. savignyi* derived from one female. (*a*) Relatively broad as compared with (*b*) (similar differences have been granted specific status).

adults (Figs. 2, 3) and in surface markings. On the basis of Schulze's classification it would not have been difficult to separate the offspring of one female into four or five species or at least subspecies. In one specimen a bridge was present above the parma but not in others. According to Schulze the absence or presence of a bridge is of prime specific importance.

Breeding experiments proved that *H. savignyi* and *H. savignyi pusillum* are identical. The latter name was applied by Schulze to a small tick 4 × 3 mm., feebly chitinized and with a parma distinctly round in contrast to the very much larger *H. savignyi*, in which the parma is triangular. In addition, there are very great differences between dwarf and normal

specimens, not only in size, but also in the number of sensory elements in the dorsal porosa, so much so that on the basis of material captured in the field we concluded that they represented two distinct species. Breeding experiments in the laboratory clearly showed that we were dealing with a single species subject to very wide variations. Pusillum types were produced from normal individuals and vice versa.

Since in many cases macroscopic examinations proved to be insufficient and even misleading, we searched for microscopic characters which might be of diagnostic value.

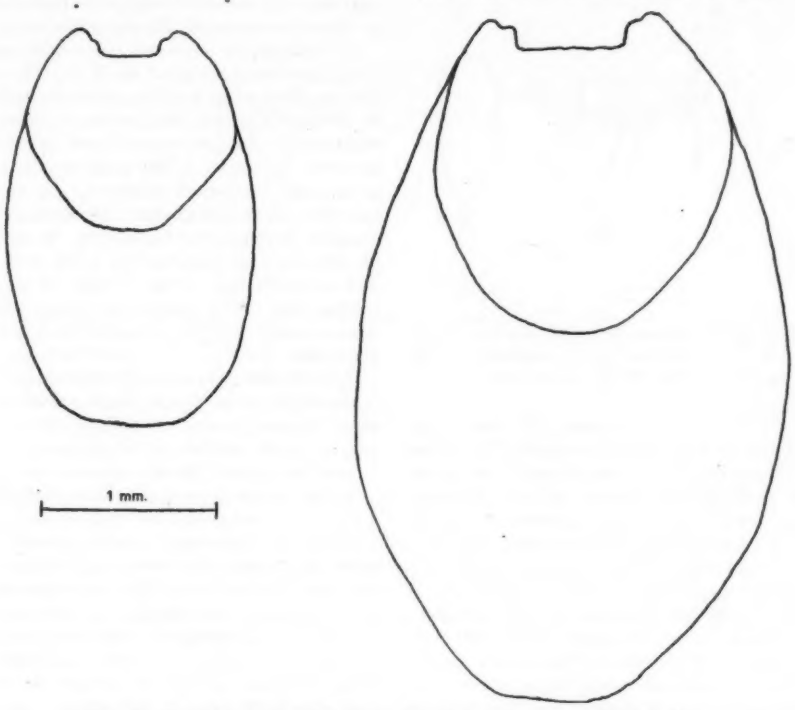

Fig. 3. *H. savignyi*, unfed females, showing range of variation in size.

The following method was employed. Ticks were placed in lactophenol for 24 hr. and then transferred to 2% potash for 3–5 days. They were then passed through water, alcohols, and clove oil and mounted in Canada balsam.

The following characters were found valuable for diagnostic purposes in *Hyalomma* and will probably prove helpful in other genera.

(1) The cuticle in the vicinity of the stigmata. In two species, *H. schulzei* and *H. impressum*, the cuticle is pilose in this region in both sexes but not in other Palestinian species.

(2) The shape of the female genital aperture. *This is the most valuable character of all and was found constant* in the offspring of individual females of *H. savignyi*, *H. detritum* and *H. dromedarii*. After oviposition the genital aperture is distorted and has no further diagnostic value.

(3) Throughout the cutis there are embedded varying numbers of minute sense organs often resembling arrow-heads in shape. Unfortunately, their number is not constant in any region. In the anterior border of the scutum there are narrow longitudinal tunnels (probably the ducts and secretory cells) and their number, although subject to variations, is sufficiently characteristic for each species to be of some diagnostic value (Fig. 8).

(4) The dorsal porosa varies surprisingly in size and number of sensory elements. (In *H. savignyi*, ♀, from 45 to 110 μ in diameter; and the number of sensory elements varies from 30 to 87 in normal specimens, and from 12 to 30 in pusillum forms.) Nevertheless, the dorsal porosa is of some diagnostic value, and in most specimens of *H. detritum*, ♀, it is typically elliptical. In other species it is circular or almost circular.

(5) The position (though not the shape) of the subanal plates which is typical in *H. dromedarii*.

(6) The shape and relative width of the palps (Fig. 4).

(7) Pigmentation of the parma.

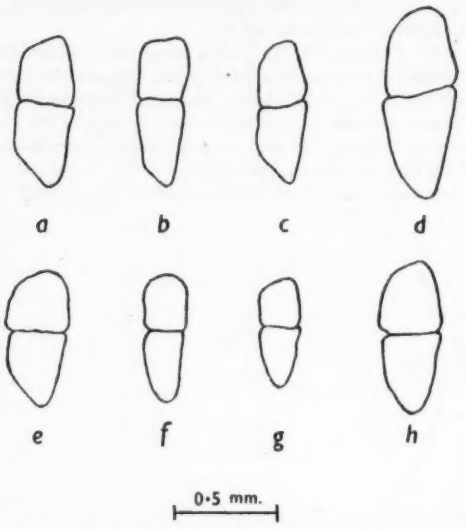

Fig. 4. Palps: (*a*) *H. dromedarii*, (*b*) *H. detritum*, (*c*) *H. savignyi*, (*d*) *H. marginatum*, (*e*) *H. dromedarii*, (*f*) *H. detritum*, (*g*) *H. savignyi*, (*h*) *H. marginatum*.

Size in the adults is so variable that there is no place for it among diagnostic characters. The colour markings on the legs were found helpful only in the case of *H. detritum* (the presence of a longitudinal yellow band on the legs in the male; in the female this band may be almost indiscernible). The character of the first coxa is decisive only in *Hyalomma* (*Hyalommasta*) *aegyptium*, ♂ and ♀, a parasite of reptiles (we found specimens on several occasions on hedgehogs and in one instance on the little owl *Athene noctua*). The females of Palestinian species are quite easy to diagnose on the basis of two combined characters, i.e. the genital aperture and the cuticle near the stigmata. The males are more difficult. Since breeding experiments have clearly shown the wide limits of variation within some species, it is obviously difficult as yet to associate many of the current names with specific biological entities. It will be necessary to re-examine type specimens with regard to constant characters before the nomenclature can be cleared up. There are, however, a number of species in which one or both sexes can be diagnosed owing to some individual taxonomic peculiarity.

Hyalomma aegyptium (Linn., 1764). Schulze (1930) clarified the position and synonymy of this species and showed that the name *H. aegyptium* cannot be applied to any *Hyalomma* of domestic animals. He examined a specimen labelled *Acarus aegyptium* by Linnaeus and found the indentation in the first coxa relatively shallow as compared with that in *Hyalomma* of domestic animals; and the outer limb of the first coxa shorter than the inner, a character typical of the species found on reptiles which Koch had named *H. syriacum*. The latter name is therefore a synonym of *H. aegyptium* (Schulze created the subgenus *Hyalommasta* on the above characters of the first coxa).

H. dromedarii, ♂, can be diagnosed because the subanal plates are external to the long axis of the anal ones. *H. schulzei* is unique in that the stigmata, as already mentioned, are feminine in type.

The specimens collected from domestic animals which had been accepted as *H. aegyptium* till 1930 are now, following Schulze, generally referred to as *H. savignyi* (Gervais, 1844) on the basis of figures first published by Savigny in 1827 and copied by Gervais in 1844. In view of the difficulty in identifying living and preserved material, an identification based on old drawings and in the absence of original material is somewhat hazardous. In this paper we use the name *H. savignyi* for a tick with yellow to yellow-brown legs, with a white or almost white parma, and other characters which will be mentioned later. It is the commonest tick of cattle in Palestine.

The following can also be identified: *H. detritum*, ♂ (because of the yellow longitudinal band on the legs), *H. marginatum*, ♂ (because of the pigmented parma), and probably *H. impressum*, ♂ and ♀ (the scutum is densely pitted with small excavations).

In the material examined from Palestine we can associate seven species with names and descriptions found in the literature; in four of these, *H. dromedarii*, *H. savignyi*, *H. detritum*, and *H. marginatum*, we were able to detect the corresponding females and establish their diagnostic characters by examining the offspring of individual females; and in the case of *H. schulzei*, ♀, we established the diagnosis of the female on the cuticle near the stigma. We can, therefore, name seven 'certain' species.

(1) *Hyalomma* (*Hyalommasta*) *aegyptium* (Linn., 1764). A parasite of reptiles, also found on hedgehogs. Readily diagnosed by the character of the first coxa.

(2) *Hyalomma savignyi* (Gervais, 1844). This is the commonest *Hyalomma* found on cattle. The legs are yellowish to brown without longitudinal bands. The number of 'tunnels' on the anterior border of the scutum varies from 20 to 35 in the female (in most specimens 25 to 30), and from 15 to 25 in the male. The dorsal porosa is round, from 45 to 110 μ in diameter and the number of elements varies from 12 to 30 in small specimens; and from 30 to 87 in large ones. A small dorsal porosa is associated with a small anterior porosa in the female. The genital

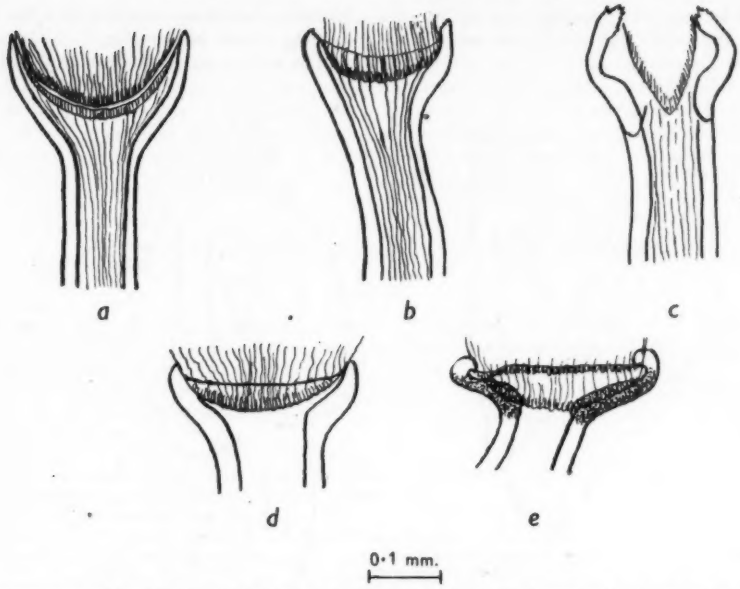

Fig. 5. Genital aperture of female: (*a*) *H. detritum*, (*b*) *H. savignyi*, (*c*) *H. dromedarii*, (*d* and *e*) *H. marginatum*.

aperture in the female (Fig. 5*b*) forms a smooth shallow concave basin. The size of the ungorged adult varies from about 2·7 to 7 mm. in length. As already emphasized, the size is so variable as to be useless for diagnostic purposes (Fig. 3).

(3) *H. detritum* Schulze, 1919. The male is characterized by yellow longitudinal band on legs. The number of 'tunnels' in the anterior part of the scutum varies from 15 to 20 in the female and 10 to 15 in the male. The dorsal porosa in many females is elliptical ($70 \times 56\,\mu$). The genital aperture in the female (Fig. 5*a*) is much deeper than in *H. savignyi* and forms a semicircle. The palps are relatively long and narrow (Fig. 4*b*).

(4) *H. dromedarii* (Koch, 1844). The male is recognized by the position of the subanal plates. The genital aperture of the female is typical (Fig. 5*c*), being cone shaped with steep slanting sides. The palps are relatively short and wide (Fig. 4*a*).

(5) *H. marginatum* (Koch, 1844). The number of 'tunnels' in the anterior margin of the scutum varies from 22 to 40 in the male (in most specimens between 25 to 30) and from 30 to 50 in the female (in most specimens more than 30). The parma of the male is pigmented. The genital aperture in the female is typical, the anterior part being almost flat (Fig. 5 *d*, *e*).

(6) *H. schulzei*. The male, as already mentioned, is unique in that the stigmata approach the feminine type and the cuticle in their vicinity is pilose. The latter character is also present in the female (Fig. 6).

Fig. 6. *H. schulzei*. Pilose cuticle in vicinity of stigma.

This species is rare in Palestine and up to the present we have found only one female and twelve males.

stigmata is comma shaped and the cuticle in its vicinity is also pilose (Fig. 7*a*). This species is rare and so far we have found only two males and two

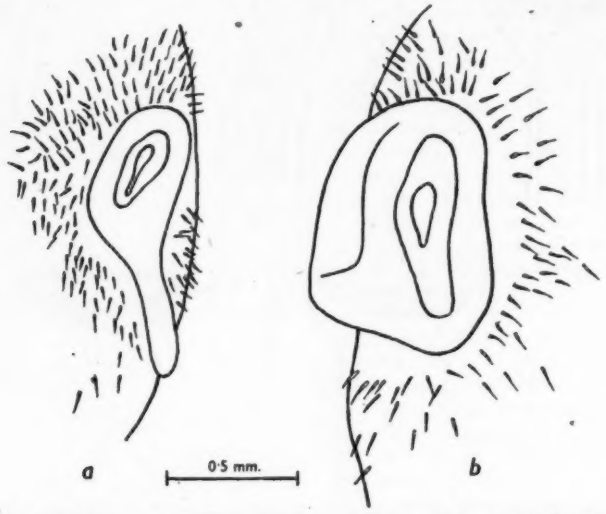

Fig. 7. *H. impressum*. Pilose cuticle near stigma: (*a*) male, (*b*) female.

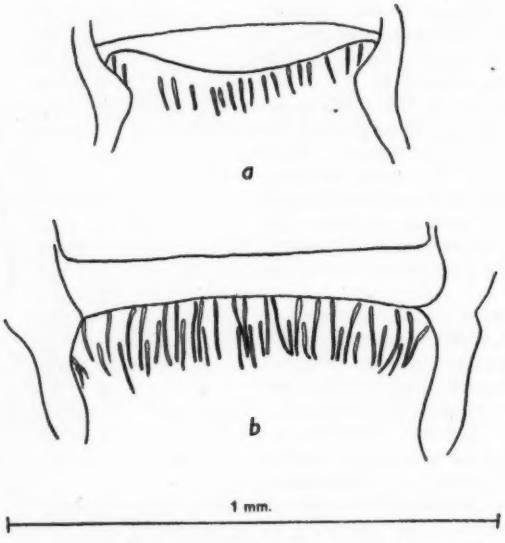

Fig. 8. (*a*) Anterior border scutum of *H. detritum*, ♀, showing 'tunnels'; (*b*) Anterior border of scutum of *H. marginatum*, ♀, showing 'tunnels'.

(7) *H. impressum*. The scutum is densely pitted with numerous excavations. The cuticle in the vicinity of the stigmata in the female is pilose, but less so than in *H. schulzei* (Fig. 7*b*); the male females. We have compared Palestinian material with specimens from South Africa and found them indistinguishable.

We have not carried out transmission experiments

with any of the above species, but we think *H. detritum* is a probable carrier of *Theileria annulata*, because it is similar to *Hyalomma mauritanicum* which is a proved carrier in Algeria (Schulze considers that *H. mauritanicum* is a subspecies of *H. detritum* and refers to it as *H. detritum mauritanicum*. Senevet thinks the two species are distinct).

SUMMARY

The determination of *Hyalomma* sp. is discussed. In the material examined from Palestine the genital aperture of the female, the cuticle in the proximity of the stigmata and the number of 'tunnels' in the anterior border of the scutum are characters which facilitate diagnosis.

KEY TO THE GENUS *HYALOMMA* IN PALESTINE

FEMALES

1 (*a*).	External process of first coxa shorter than internal. Subgenus *Hyalommasta*.	*H. aegyptium*
(*b*).	External and internal processes of first coxa approximately equal. Subgenus *Hyalomma*.	2
2 (*a*).	Cuticle in vicinity of stigma markedly pilose.	3
(*b*).	Cuticle in vicinity of stigmata not markedly pilose.	4
3 (*a*).	Cuticle in vicinity of stigmata densely pilose.	*H. schulzei*
(*b*).	Less densely pilose.	*H. impressum*
4 (*a*).	Anterior part of genital aperture shallow.	5
(*b*).	Anterior part of genital aperture deeply convex posteriorly.	6
5 (*a*).	Anterior part of genital aperture convex posteriorly.	*H. savignyi*
(*b*).	Anterior part of genital aperture almost flat.	*H. marginatum*
6 (*a*).	Anterior part of genital aperture cup shaped and almost a semicircle.	*H. detritum*
(*b*).	Anterior part of genital aperture cone shaped, narrow, with sloping sides.	*H. dromedarii*

MALES

1 (*a*).	External process of first coxa shorter than internal one. Subgenus *Hyalommasta*.	*H. aegyptium*
(*b*).	External process of first coxa approximately as long as internal one. Subgenus *Hyalomma*.	2
2 (*a*).	Cuticle near stigmata markedly pilose.	3
(*b*).	Cuticle near stigmata markedly not pilose.	4
3 (*a*).	Stigmata feminine in type.	*H. schulzei*
(*b*).	Stigmata comma shaped.	*H. impressum*
4 (*a*).	Parma pigmented.	*H. marginatum*
(*b*).	Parma not pigmented.	5
5 (*a*).	Subanal plates external to longitudinal axis of anal plates.	*H. dromedarii*
(*b*).	Subanal plates under anal plates and not external to their longitudinal axis.	6
6 (*a*).	Legs with yellow longitudinal band.	*H. detritum*
(*b*).	Legs yellowish without longitudinal band (palps wider and shorter than in *H. detritum*).	*H. savignyi*

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THE TECHNIQUE FOR BREEDING PURE-LINE CULTURES OF THE BLOW-FLY (*CALLIPHORA ERYTHROCEPHALA*)

By P. TATE, *From the Molteno Institute, University of Cambridge*

(With 3 Figures in the Text)

During genetical studies of a white-eyed mutation of the blow-fly (*Calliphora erythrocephala*) it was found that in order to obtain reliable data from breeding experiments the most stringent precautions had to be taken to prevent contamination of the breeding pabulum by stray flies of the same or other species. As the maintenance of pure-line cultures may be of importance in other work, such as standard tests of insecticides, the methods devised for the genetical studies should be of interest to all workers concerned with the breeding of this fly.

The oviposition habits of the blow-fly are such that it is difficult to prevent contamination of cultures unless special methods are adopted which, in some respects, must be more elaborate than the technique used in bacteriology. Gravid females are attracted towards meat, especially if it is decomposing, and will deposit eggs in the vicinity of the meat if they are unable to reach it. The first-stage larvae, which hatch from the eggs in 24–48 hr., can insinuate themselves through minute apertures and will traverse long distances to reach the food. In the past, non-recognition of this fact led Parker

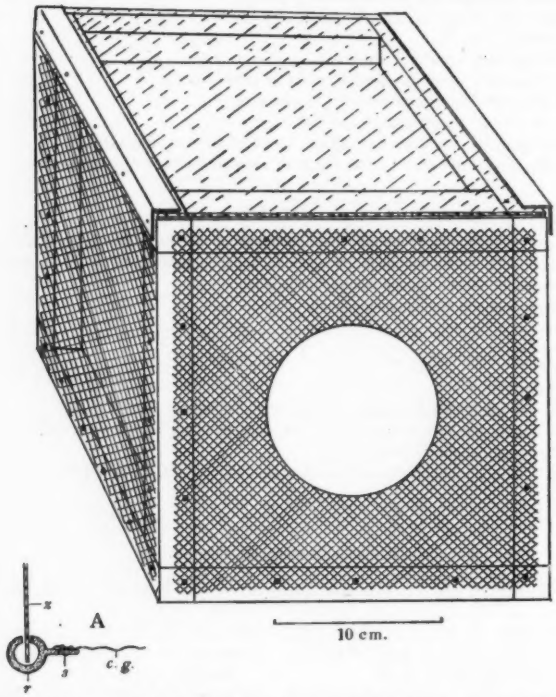

Fig. 1. Perspective view of the cage used for the breeding experiments. A, a sectional view of the manner in which the gauze sleeve is fixed to the front of the cage; *c.g.* cotton gauze sewn to the flange, *s*, of the rubber draught-excluder strip, *r*, the cylindrical border of which is slit to take the edge of the perforated zinc, *z*, surrounding the central opening.

(1922) to describe paedogenetic multiplication in the blow-fly. His results were disproved by Keilin (1924) who pointed out the pitfalls in such work and devised a technique for breeding the flies with the minimum danger of contamination by the use of water barriers surrounding the vessels containing the cultures of larvae. The closing of culture vessels by means of cotton wool or gauze covers, such as suffices for work on *Drosophila*, is quite insufficient to protect cultures of blow-flies from chance contamination.

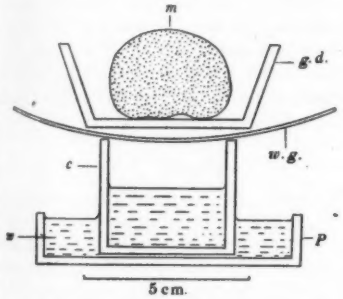

Fig. 2. Optical view of the appliance used for protecting the meat in the breeding cages from external contamination. *P*, petri dish full of water, *w*, in the centre of which stands a short glass cylinder, *c*, supporting a watch-glass, *w.g.*, upon which rests a glass dish, *g.d.*, containing the meat, *m*.

THE BREEDING CAGE

A very convenient cage for breeding has been devised (Fig. 1). It consists of a wooden frame, about 25 cm. square, made of 2 cm. square battens. The floor is formed of zinc and the back and sides are covered with perforated zinc. The front is also covered with perforated zinc but has a hole 10 cm. in diameter in the centre. To this hole is fixed a cotton gauze sleeve about 20 cm. long. The sleeve is sewn to the flange of a circle of rubber draught-excluder strip which has a slit made in the external circumference of the cylindrical edge. This slit enables the sleeve to be detachably affixed to the zinc front. The edge of the zinc border of the central opening is inserted in the slit so that one lip of the rubber cylinder lies within the cage and the other without it, thus gripping the zinc and firmly attaching the sleeve to the cage (Fig. 1 A). By bending the rubber strip inwards it can be disengaged from the zinc and the sleeve is easily removed for washing. The top of the cage is formed of a square of glass sliding in zinc runners. One of these cages holds 50–100 flies for breeding.

IMAGINES

Food consisted of meat, preferably fresh horse flesh. Owing to wartime difficulties an adequate supply of this could not always be obtained and sometimes dead experimental animals, rats, guinea-pigs or chicks, had to be used. The meat was always plunged into boiling water to destroy contaminating eggs or larvae and then cooled before being put in the cage or fed to larvae. Forceps or other instruments were always dipped in boiling water before and after use to prevent eggs or young larvae being carried over from one culture to another.

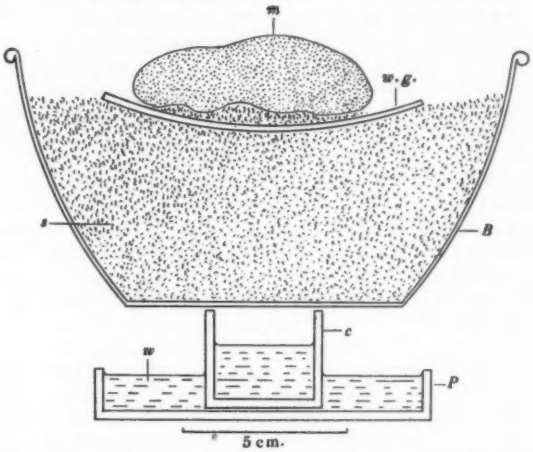

Fig. 3. Optical view of the appliance used to prevent contamination of the cultures during the larval stages. The whole appliance was kept in a gauze cage with a glass top. *B*, enamel-ware bowl; *s*, sawdust; other lettering same as in Fig. 2

A vessel full of water was kept in the cage and a disk of cork was floated on the surface of the water to prevent the flies from being drowned when drinking. The flies were given dextrose in addition to meat. The dextrose was put into a small petri dish and moistened just sufficiently to cake it but not enough to make it wet and sticky. When eggs were wanted for breeding purposes the meat was put in a glass dish which was placed upon a glass support standing in a petri dish of water so that stray, wandering larvae could not reach it without crossing the water barrier. To prevent the flies from

being trapped in the exposed water, a large watch-glass was placed beneath the dish containing the meat so that it projected beyond the petri dish and prevented the flies from falling on to the water (Fig. 2).

LARVAE AND PUPAE

The larvae were reared on meat which rested on a little sawdust in 12 cm. watch-glasses which were placed on top of sawdust about three-quarters filling a 15 cm. enamelled bowl. This bowl was supported on a glass vessel standing in the centre of a petri dish full of water (Fig. 3) and the whole apparatus was kept in a fly-proof cage with gauze sides and a glass top. It is important that breeding cages should have closed tops as eggs laid on gauze or netting might drop on to the larval food. The meat was examined daily and more added as required, but the offensive smell from cultures could be reduced greatly by giving only as much meat as the larvae could utilize so that not much bacterial decomposition took place. The fully fed larvae left the meat and buried themselves in the sawdust to pupate. Pupae were collected by sieving the sawdust. The pupae, or pupating larvae, were transferred to fresh sawdust in glass dishes (11 cm. by 5 cm.) with glass covers, and when imagines emerged they were lightly anaesthetized with ether or chloroform, sorted and counted. The emergence of a batch of pupae usually extended over 2 or 3 days; and of the imagines that emerged the first day, the great majority were males, and of those emerging later the majority were females. About one hundred larvae is a convenient number to rear in the vessels described. The pupae had to be kept in glass dishes with closely fitting lids to prevent their being parasitized by Hymenoptera which caused great mortality when pupae were kept in gauze-covered vessels. The flies were kept in a warm room at a temperature of 20–21° C. which proved very suitable for breeding. At this temperature eggs hatch in about 24 hr.; larval life is about 6 days, pupal life 12–14 days; and imagines begin to lay about 7–10 days after emergence. Thus, the complete cycle from egg to egg takes about 4 weeks. Unfortunately, in summer the temperature of the insect room could not be kept as low as 21° C. in very hot weather and breeding was more difficult and erratic. On occasions the electric thermostatic control failed and the temperature rose to 30–35° C. overnight for a period of unknown duration. Subjection to such high temperature interfered seriously with breeding as it appeared to have a sterilizing effect and eggs failed to hatch for long periods after the rise in temperature. It has not been determined what stage, egg or sperm, is affected by the high temperature.

A relative humidity of about 80% is satisfactory for breeding, but the flies thrive at lower humidities although they do not live so long and they tend to lose their wings quickly through fraying at the edges. A breeding cage containing 50–100 flies continued to yield eggs for 4–6 weeks but, by the end of that time, most of the flies had died, and consequently breeding stocks were renewed at intervals of 5–6 weeks. It was found that single pairs did not mate readily or live long in small containers so that, owing to shortage of space, only a little work with single-pair mating could be done. More usually the flies were allowed to mate in the standard breeding cages and then females were isolated and the eggs collected from them. Such isolated females sometimes continued to lay after their spermatozoa had all been used or had become non-viable because infertile batches of eggs were laid after many fertile batches had been obtained from the same female.

SUMMARY

1. Pure-line breeding of *Calliphora erythrocephala* is difficult owing to the fact that gravid females will oviposit at a distance from decomposing meat and the newly hatched larvae will crawl long distances and pass through minute openings to reach the food.

2. A simple and convenient cage for breeding blow-flies is described.

3. To obtain eggs free from contamination meat is plunged in boiling water before being put in the cages; and the vessel in which it is placed is supported on a glass cylinder standing in a dish of water.

4. The larvae are fed on horse-meat placed upon sawdust in a large watch-glass resting upon sawdust in a bowl. The bowl rests upon a support surrounded by water.

5. Pupation occurs in the sawdust beneath the watch-glass and puparia are separated from the sawdust by sieving.

6. Pupae are kept in glass dishes with closely fitting covers to prevent parasitism by Hymenoptera.

7. At a temperature of 21° C. the complete life cycle takes about 1 month.

I wish to thank my assistant, Miss Elisabeth Stanley, for her help in the tedious work of maintaining the many pure-line cultures.

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THE RESPIRATORY METABOLISM OF PARASITIC NEMATODES

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(With 2 Figures in the Text)

INTRODUCTION

The respiratory activity of *Ascaris lumbricoides* and *Parascaris equorum* has been examined by many workers (see Laser, 1944, for references). The results of such investigations, however, cannot be used as a basis for generalizations concerning parasitic nematodes as a group, for these species are highly atypical in respect to size. In order to obtain information of wider implication, the present investigation has included a wide group of nematodes in several stages of development, viz.:

(1) *Nippostrongylus muris*, adults from the small intestine of the rat: infective larvae and eggs.

(2) *Neoaplectana glaseri* (a parasite of the tissues of the Japanese beetle, Glaser, McCoy & Girth, 1940), larvae and adults grown *in vitro*.

(3) *Nematodirus* spp., adults from the small intestine of the sheep.

(4) *Haemonchus contortus*, third stage larvae and eggs.

(5) *Ascaridia galli*, adults from the small intestine of the chicken.

From study of these forms certain generalizations on the nature of respiratory metabolism of parasitic nematodes are advanced.

BIOLOGICAL MATERIALS

Nippostrongylus muris adults were obtained from the small intestines of rats which had been starved for several hours before being killed. The parasites were largely freed from debris by sedimentation in 0·9% saline and were then picked out with a Pasteur pipette. They were washed at least five times with saline before use. As a rule, it took several hours to prepare the material for examination.

Infective larvae were obtained free from debris after they had migrated from faeces cultured on damp filter-paper. They were washed with several changes of sterile water before examination. Eggs were collected from fresh faeces by centrifugation and flotation in 50% sucrose. They were washed well with water before use. Eggs treated in this manner developed normally if returned to a suitable culture medium. It was not possible to remove all contamination from the eggs and in some instances at least 5% of the weight of the material used for examination was due to foreign matter.

Neoaplectana glaseri was grown on sterile sheep- and rabbit-liver slices supported on neutral agar in Roux bottles. The mixed populations of larvae and adults were removed from the cultures with 0·5% saline and washed at least eight times before use.

Nematodirus adults were collected individually from ingesta, and were washed with 0·9% saline. The parasites were examined manometrically within 4 hr. of the killing of the host.

Ascaridia galli adults were also washed in 0·9% saline. Experiments were commenced within 30 min. of the killing of the host.

Haemonchus contortus infective larvae were obtained from faecal cultures in the ordinary manner, and were washed with water before use. Eggs were concentrated by centrifugation and flotation in 50% sucrose. They were cleaned further by differential flotation in sucrose solutions (a method developed by Mr H. Whitlock of this Laboratory) and washed with water. The amount of contamination in egg suspensions varied greatly, but in some instances probably amounted to less than 1%. It was found that the respiratory activity of adult parasites was not obviously impaired when they were kept in cold saline for periods twice as long as those needed for the routine preparation of material used in these investigations. Fortunately, the large parasite *Ascaridia galli*, which was most rapidly affected after removal from the host, could be prepared for investigation in the minimum of time. The results obtained may therefore be considered to apply to fresh material immediately after removal from the host.

METHODS

Respiratory activity was followed in Barcroft, or in open-type Warburg manometers. For *Ascaridia galli* Warburg vessels, of about 12 ml. volume, were used. To allow the use of material collected in the minimum time, smaller vessels of about 5 ml. were used in the examination of small parasites. Oxygen uptake was determined by the direct method of Warburg (1926), the carbon dioxide being absorbed by potassium hydroxide. In respiration experiments

in presence of cyanide, suitable potassium cyanide-potassium hydroxide mixtures (Umbreit, Burris & Stauffer, 1945) were used to prevent the distillation of cyanide from the medium. Potassium cyanide was neutralized before addition. Except where otherwise stated, the gas phase used was air. Q values (μl. of gas taken up or given off per hour per mg. dry weight of tissue used) were calculated from figures of gas exchange taken during the first 20 min. of the experiment.

With the exception of *Neoaplectana glaseri*, for which 0·5% saline was used, adult parasites were examined in 0·9% saline. Infective larvae and eggs were examined in water.

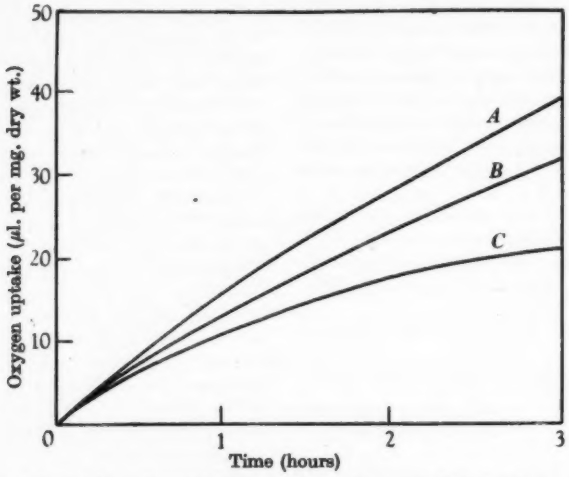

Fig. 1. Oxygen uptake (μl./mg. dry wt.) of *Nippostrongylus muris* infective larvae 2 days old (*A*), 6 days old (*B*) and 12 days old (*C*).

RESULTS

The eggs of *Haemonchus contortus* and *Nippostrongylus muris*, which were examined at 30° C., showed a similar respiratory activity, constant for periods of at least 1 hr. The more highly developed eggs of *Haemonchus contortus* gave a higher Q_{O_2} than freshly passed eggs (see Table 1). Since the washings from eggs used in the experiments showed a negligible oxygen uptake and dry weight determinations of the eggs included a certain amount of contaminating plant material from the faeces, the Q_{O_2} values given are probably slightly lower than the true figures. Q_{CO_2} values for both species were consistently small and gave an R.Q. of about 0·6. Histological examination showed that the eggs contained glycogen (Best's Carmine Stain) and a little fat (Sudan III Stain). Cyanide inhibited the respiration of both *Nippostrongylus muris* and *Haemonchus contortus* eggs by about 70% (0·01M potassium cyanide, see Table 3).

Third-stage larvae of *H. contortus* and *Nippostrongylus muris* were examined manometrically at 30° C. The Q_{O_2} of fresh *N. muris* larvae was high, but fell off rapidly as the larvae aged (see Fig. 1). The R.Q. of both species was found to be in the region of 0·7 (see Table 2). Cyanide (0·001M) caused a decrease in oxygen uptake of only 34%. However, the inhibition in 0·01M cyanide rose to 92%. The character of the respiration of *N. muris* larvae was not changed after exsheathment in hypochlorite solution either at 30 or 37° C. *Haemonchus contortus* larvae gave a somewhat lower Q_{O_2} than fresh *Nippostrongylus muris* larvae, though the R.Q. was again about 0·7.

Table 1. *Respiratory activity of* Haemonchus contortus *eggs at different stages of development*

Stage of development	Q_{O_2}	Q_{CO_2}	R.Q.
Early morula	− 9·7	+5·8	0·60
Late blastula	−10·7	+6·2	0·58
Fully developed larvae	−12·6	+8·1	0·64

Table 2. Q_{O_2}, Q_{CO_2} *and* R.Q. *of* Nippostrongylus muris *infective larvae of different ages*

Age of larvae	Q_{O_2}	Q_{CO_2}	R.Q.
1 day	−18·4	+13·5	0·73
4 days	−13·0	+ 9·6	0·74
12 days	− 9·2	+ 6·2	0·68

Fat was present in large amounts in the intestinal cells of fresh *Haemonchus contortus* (Rogers, 1940) and *N. muris* larvae. Only small amounts of glycogen could be detected by histological means.

The results obtained with adult parasites are shown in Table 4. *Neoaplectana glaseri* was examined at 30° C., other species at 37° C. Some variations were noted for the R.Q.'s, but the Q values did not vary to any significant extent and therefore mean figures only are shown for each species. The respiration of *Ascaridia* and *Nematodirus* was little affected by increasing oxygen tensions: the R.Q.'s fell slightly and the Q_{O_2} rose very little.

The inhibition of oxygen uptake by 0·01 M and 0·001 M potassium cyanide is shown in Table 3.

specific gravity of the parasite tissues has been considered to be of a constant value. (The dry-matter content of different adult species varied from 20·0 to 22·8 %, the lower figures referring to small worms from which it was difficult to remove all extraneous water for the determination of wet weights.) The relative surface area of given weights of different adult and larval parasites was determined by treating the worms as long continuous cylinders. The diameters of the parasites were determined by means of a micrometer eyepiece. Males and females

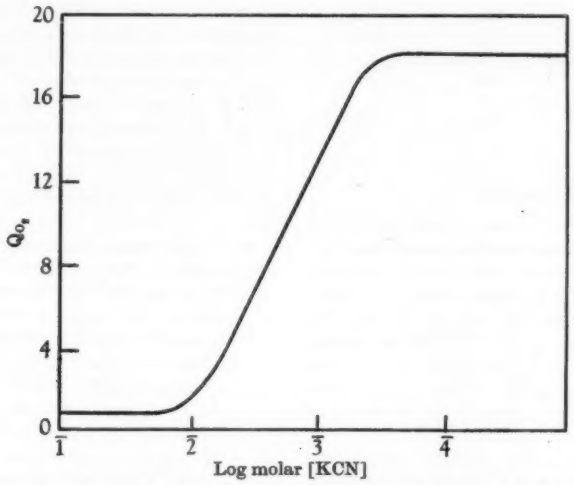

Fig. 2. Oxygen uptake (μl./hr./mg. dry wt.) of *Nippostrongylus muris* infective larvae in potassium cyanide of different concentrations.

Table 3. *Inhibition of the oxygen uptake of several parasitic nematodes (adults, eggs and larvae) by* 0·01 M *and* 0·001 M *potassium cyanide*

Species	Percentage inhibition in KCN 0·01 M	0·001 M
Haemonchus contortus (eggs)	84	68
Nippostrongylus muris (infective larvae)	92	34
N. muris (adults)	54	36
Nematodirus spp. (adults)	68	64
Nematodirus spp. (adults, brei)	72	65
Ascaridia galli (adults)	68	62
Neoaplectana glaseri (larvae and adults)	91	96

With the exception of the nematode eggs, the organisms used in this investigation were of a similar shape but varied greatly in size. The surface area of a given weight of tissue therefore varied also, and respiratory activity might best be examined per unit surface area rather than on a weight basis. For the purpose of determining relative surface areas the

Table 4. Q_{O_2}, Q_{CO_2} (*mean values obtained from at least three determinations*) *for adult parasitic nematodes when examined in saline solutions*

The limits of the variation for R.Q. values are shown; mean values are given within brackets

Species	Q_{O_2}	Q_{CO_2}	R.Q.
Ascaridia galli	− 2·5	+2·3	0·85 − 1·01 (0·96)
Nematodirus spp.	− 5·1	+3·5	0·55 − 0·69 (0·66)
Nippostrongylus muris	− 6·8	+4·9	0·64 − 0·76 (0·69)
Neoaplectana glaseri	−12·6	+7·5	0·55 − 0·67 (0·59)

were measured at several points along their length and a mean value determined according to the proportion of the sexes present. The diameters thus obtained were only rough approximations, particularly in the case of *Nematodirus* where the thickness varied greatly along the length of each female worm. For the purpose of calculating the surface area, the nematode eggs were treated as spheres. The relative surface areas of the different parasites and the

relative respiratory activities calculated on a surface area basis are given in Table 5.

Table 5. *Relative oxygen uptake for parasitic nematodes (adults, eggs and larvae) when calculated on a surface area basis*

For further explanation see text

Species	Relative surface area	Relative rate of oxygen uptake
Haemonchus contortus (eggs)	50	0·8
H. contortus (larvae)	100	0·8
Ascaridia galli (adults)	1	10·0
Nematodirus spp. (adults)	12	1·7
Nippostrongylus muris (adults)	19	1·4
Neoaplectana glaseri (larvae and adults)	44	1·1

DISCUSSION

The Q_{O_2} values listed in Tables 1, 2 and 4 indicate that all the organisms concerned had a higher oxygen uptake than *Ascaris* (Laser, 1944). With the exception of *Ascaridia*, the Q_{O_2} values were also higher than those of *Eustrongylides ignotis* larvae (von Brand, 1942) and the small *Trichinella spiralis* larvae (Stannard, McCoy & Latchford, 1938). Most of the parasites had a higher oxygen uptake than *Paramecium* (Krogh, 1941) though less than that of some mammalian tissues (Krebs, 1939).

The adoption of a parasitic mode of life involves the exsheathment of infective larvae and a change in environmental temperature. It is notable that the nature of the respiration of *Nippostrongylus muris* and *Haemonchus contortus* infective larvae was not changed by exsheathment whether they were examined at 37 or 30° C.

The variation in Q_{O_2} values for different species was not completely eliminated when oxygen uptake was calculated on a surface area basis, whether the area was determined by measurement (see Table 5) or estimated on the assumption that it was proportional to $W^{\frac{2}{3}}$ (the square of the cube root of the weight). It is clear that the larger species of nematodes give unduly high results and Krüger's (1940) relation between respiratory activity and external surface area for *Ascaris lumbricoides* of different sizes does not hold when comparing different species.

In view of the large tissue-glycogen reserves in *Ascaridia galli*, the R.Q. of about 1, found for this species, is not unexpected. Similar R.Q. values have been found for the larval tissue parasites *Eustrongylides ignotis* (von Brand, 1942) and *Trichinella spiralis* (Stannard *et al.*, 1938). The small adult forms used in the present investigation, however, all gave low R.Q. values ranging from about 0·6 to 0·7 (see Table 4). It is perhaps significant that the large parasite *Ascaris lumbricoides* has an R.Q. of 0·6 when examined at high oxygen tensions (Laser, 1944). Further, results obtained with *Ascaridia galli* were similar to those obtained with small parasites when the oxygen tension was increased. Increases in oxygen tension did not appreciably affect the magnitude of respiration of the smaller parasites. There is some suggestion then that, given adequate oxygen saturation, a low R.Q. is not uncommon in nematode parasites. Some nematode eggs evidently show this characteristic also. Ammonia production by certain protozoa gives rise to an apparent R.Q. of low value (Specht, 1934). However, no appreciable amounts of acid absorbable gas were produced by the nematodes used in the present experiments. Low R.Q. values have been found for some other invertebrates (e.g. see Gilmour, 1940).

The Q_{O_2} values given above were determined, as is usual, in the absence of carbon dioxide and, as the respiratory activity may be affected by the carbon dioxide tension (Laser, 1942), the determination of the R.Q. by the direct method of Warburg may give misleading results in instances such as this, where carbohydrate utilization is affected by the presence of carbon dioxide-bicarbonate buffer (Rogers, unpublished). It is quite probable, however, that in certain parasitic nematodes the metabolites may not be oxidized as far as carbon dioxide and water, in which case non-gaseous end products may replace some of the carbon dioxide normally formed. In addition to the glycogen reserves commonly found in adult nematode parasites, considerable amounts of fat were demonstrable by histological means. The existence of an active fat metabolism would also tend to lower the R.Q's.

Infective larvae, which largely metabolize fat (Rogers, 1939, 1940), would be expected to have a low R.Q., as was found. The low R.Q. values were also obtained when larvae were examined by the indirect method of Warburg (1924). In organisms such as infective larvae, enclosed in a sheath and very susceptible to water loss, the importance of metabolic water would be greatly increased and dependence on fat metabolism might have considerable survival value.

It is clear that the cytochrome system plays some role in oxygen transport when parasites are examined under the conditions used for these experiments.

The applicability of results obtained when parasites, taken from an environment low in oxygen such as intestinal contents, are examined in solutions in equilibrium with air, is open to question. Nevertheless, the respiration of *Neoaplectana glaseri* was similar to that of the other adult parasites and it is probable that, given access to oxygen from the host tissues, respiration involving endogenous substrates would follow a course indicated by the presen

experiments. It would appear that nematode eggs respire in a manner similar to that of adult parasites.

SUMMARY

1. Q_{O_2} values for *Haemonchus contortus* egs varied between −9·7 and −12·6 according to the degree of development of the embryos. The R.Q. was in the region of 0·6.

2. Young infective larvae of *Nippostrongylus muris* had a high Q_{O_2} (−18·4), which fell as the larvae aged. Third stage *Haemonchus contortus* larvae gave similar results. The respiration was not affected by exsheathment.

3. Adult parasites gave Q_{O_2} values as follows: *Ascaridia galli*, −2·5; *Nematodirus* spp., −5·1; *Nippostrongylus muris*, −6·8; *Neoaplectana glaseri*, −12·6. When calculated on a surface area basis, *Ascaridia galli* had an oxygen uptake which was much higher than that of the other organisms.

4. Q_{CO_2} values were small. The R.Q. of infective larvae, which contain fat as reserve material, was about 0·72. The other forms which contained much glycogen as well as fat had R.Q. values between 0·6 and 0·7. *A. galli* was an exception in that an R.Q. of about 0·95 was obtained.

5. Potassium cyanide inhibited respiration in all the forms examined.

The author is indebted to Dr R. W. Glaser for supplying a strain of *Neoaplectana glaseri*.

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SOME NEW MYXOSPORIDIA FROM PLYMOUTH WITH A PROPOSED NEW CLASSIFICATION OF THE ORDER

By YOGENDRA R. TRIPATHI (*India State Scholar*)

From the Marine Biological Laboratory, Plymouth

(With 6 Figures in the Text)

During the examination of fish for parasitic infection nine species were found to be infected with Myxosporidia. Of these hosts four are selected for special attention in this paper, since they harboured Myxosporidia which appear to be new, and which raise points of particular interest in the systematics of the group.

Host	No. examined	Infected	Parasite	Site of infection
Solea solea	3	3	*Sinuolinea rebae* n.sp.	Urinary bladder
Zeus faber	6	4	*Leptotheca vikrami* n.sp.	Gall bladder
Gaidropsarus tricirratus	4	3	*Zschokkella russelli* n.sp.	Gall bladder
	4	2	*Sphaeromyxa balbianii* Thélohan, 1892	Gall bladder
Ciliata mustela	20	7	*Zschokkella russelli* n.sp.	Gall bladder
	20	2	*Sphaeromyxa balbianii* Thélohan, 1892	Gall bladder

The fishes supplying the material for the present paper (the common sole, *Solea solea*; the John Dory, *Zeus faber*; the Rocklings, *Ciliata mustela* and *Gaidropsarus tricirratus*) were caught near Plymouth and examined in fresh state. Other parasites from these and other fishes will be described in subsequent papers.

Whenever possible, the myxosporidians were examined and measured during life, with or without the application of the vital stains such as neutral red or methylene blue. 5% KOH was used to extrude the polar filament. Permanent slides were prepared from smears which were fixed in 2% osmic acid, or Bouin-Duboscq fluid; also by heating the slide face upwards, by passing three or four times over the bunsen flame so that the slide does not become hotter than can be borne on the back of the hand. These slides were stained with Delafield's haematoxylin or Leishmann's stain. Amongst the fixatives 2% osmic acid was found to produce less distortion of the shell valves as compared with Bouin-Duboscq fluid. Almost equally good results were obtained by heating. Leishman's stain was found preferable for spores and Delafield's haematoxylin for the trophozoites.

When Dunkerley (1921) examined fishes for Myxosporidia at Plymouth he recorded none from two soles and seven John Dorys. Of the three *Gaidropsarus tricirratus* and forty-six *Ciliata mustela*, all of the former and one of the latter harboured *Zschokkella ovata* (Dunkerley, 1921) in the gall bladder. *Sphaeromyxa balbianii* Thélohan, 1892 was found by him in the gall bladder of one other *Ciliata mustela*.

The following table shows the incidence of infection found during the recent investigations:

The above table confirms Dunkerley's findings of the low incidence of infection in the case of *Ciliata mustela*. The intensity of infection in the infected fishes was high.

The bile in all the infected fishes was more viscous and pale in colour as compared with healthy bile. In one *Gaidropsarus tricirratus*, which harboured a double infection of *Zschokkella russelli* n.sp. and *Sphaeromyxa balbianii* Thélohan, 1892, the gall bladder was whitish in appearance and the bile was thick and colourless. This change in the bile must affect the digestion of the fish and ultimately its health, but as the fishes harbour other (trematode, nematode and cestode) parasites as well, it becomes difficult to assess the total harm done by the myxosporidians. The urinary bladder of *Solea solea* apparently sustained no harm from the protozoan infection which it harboured.

DEFINITION OF TERMS USED IN DESCRIBING THE SPORES OF MYXOSPORIDIA

Considerable confusion has arisen by different authors employing different terms, some of them ambiguous, to describe various axes of the spore. The 'length' of a spore (its longest axis) in one species, may in another species represent an axis morphologically at right angles to it; so that it

order to avoid further ambiguities it is suggested that one nomenclature be strictly adhered to throughout.

The following is a summary of the existing terminology, and an attempt has been made to reduce this to a standardized form.

Among the recent authors, Davis (1917) and Kudo (1920) have defined their terms, but with different meanings. Davis measured the length along the axis *AB* (Fig. 1 *a*) (taking the spore of *Leptotheca* as an example for his definitions) while Kudo (using *Myxobolus*) measured it along the antero-posterior axis which is along the axis *CD* of Davis (Fig. 1 *a*, *c*). Fujita explained the terms he used in his 1923 paper (p. 195), but he confuses thickness ('linear distance between the farthest lateral extremities') with breadth ('transverse diameter of the spore') and his definitions are not clear. In his subsequent papers on Myxosporidia he (Fujita) has been inconsistent in the use of his terms. For example, he uses 'lateral view' in the same sense as 'front view' and 'anterior view' of Kudo. Kudo has used the term 'profile' but he did not define it. Chakravarty (1941) used the term 'profile view' to mean the 'anterior view'. The following table shows the comparison and difference between the terms used by Davis and Kudo:

	Davis	Kudo
Length	'Distance along the longitudinal axis *AB*'	'Antero-posterior diameter of the spore in the sutural plane' along the axis *CD*
Breadth	'Distance between capsular and post-capsular sides, along the transverse axis *CD*'	'Larger diameter of the spore measured at right angles to length or sutural diameter' along the axis *AB*
Thickness	'Length of the vertical axis *EF*'	'Shortest diameter at right angles to the length or sutural diameter' along the axis *EF*

Davis	Kudo	Reference to figures herein
Capsular side	= Anterior view	Fig. 1 *b*
Front side	= Front view	Fig. 1 *a*
Post-capsular side	= Posterior view	
End view	= Side view	Fig. 1 *c*
Capsular end	= Anterior end	

In the figure (Fig. 1 *a*, *b*, *c*) the different views of the spore are labelled according to Kudo's terms. For sake of convenience it is proposed to retain Kudo's terms because they are well defined and used by many subsequent authors. There is a difficulty, however, in applying these terms to the members of the family Myxidiidae Thélohan, 1892, the spores of which are different in shape and have the polar capsules separated one at each end of the spore. The terms here proposed for Myxidiidae are defined as follows (Fig. 2 *a*, *b*):

Length. The longest diameter of the spore parallel to, or at an acute angle to, the sutural axis, i.e. along the axis *XY* (Fig. 2 *a*, *b*).

Breadth. The diameter of the spore at right angles to the sutural axis (not included in the sutural plane), i.e. along the axis *PQ* (Fig. 2 *a*).

Thickness. The diameter of the spore at right angles to the sutural axis (in the sutural plane), i.e. along the axis *LM* (Fig. 2 *b*). *Sutural view:* The view which includes the greater part of the sutural line (Fig. 2 *b*). *Front view:* The view which does not include the sutural line or only a part of it, if this is sinuous (Fig. 2 *a*).

Here we cannot use the terms 'anterior' and 'posterior' as in the case of the members of other families, since with the capsules at either end, there is no suggestive morphological orientation. Also in *Sphaeromyxa* and *Myxidium*, with spindle-shaped spores, breadth and thickness will be the same.

DESCRIPTION OF NEW SPECIES

Sinuolinea rebae n.sp. (Fig. 4 *a*, *b*)

In the urinary bladder of *Solea solea* numerous spores of this species were found. Few, only very young and immature, trophozoites were observed. These were irregular in shape with granular protoplasm, which had some refringent granules in living condition. A thin hyaline ectoplasmic layer could be differentiated from the endoplasm. The size of the trophozoite was from 14 to 18 μ broad and 42 μ long. No indication of pansporoblast formation could be made out at this stage.

The spore is practically spherical, but slightly flattened at the anterior end. The shell valves are thin with a prominent sutural line, markedly sinuous, completing a figure of '8'. The diameter of the spore is 10·0–13·2 μ. The polar capsules, 2·5–3·5 μ in diameter, are prominent, spherical and each is surrounded by a large capsulogenous cell which has an ellipsoidal nucleus; the polar filament not being extruded on the application of 5 % KOH. The sporoplasm is globular in form, and granular with two centrally situated nuclei. The spore

capsule-cells are clearly seen in stained preparations.

Remarks. This form resembles *Sinuolinea dimorpha* (Davis, 1916) in shape of sutural line, and *S. capsularis* Davis, 1917 in size of the spore, but it differs from both of them in all other respects; nor can there be any confusion between the present species and the two species described by Basikalowa (1932) from U.S.S.R. (Fig. 3 *a*, *b*; and see below, p. 114).

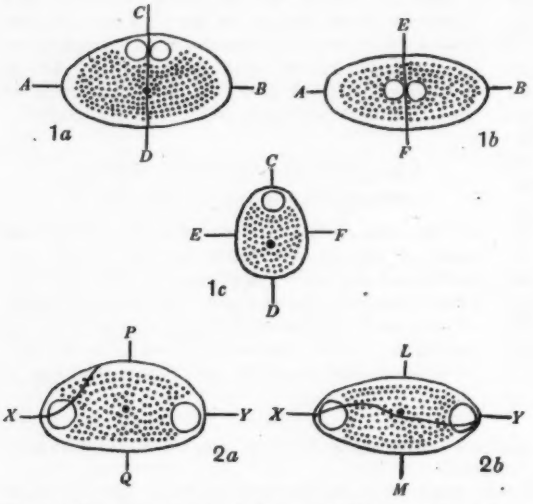

Fig. 1. Diagrams of a typical spore of the genus *Leptotheca*. (*a*) front view; (*b*) anterior view; (*c*) side view.

Fig. 2. Diagrams of a typical spore of the genus *Zschokkella*. (*a*) front view; (*b*) sutural view.

posterior end. Numerous spores were measured showing the following dimensions: length 10·3–13·7 μ, breadth 17·1–20·6 μ, thickness 8·6 μ, and diameter of the polar capsules 3·2–3·4 μ. The dimensions of a typically shaped spore were 10·3 × 18·3 μ and the diameter of the polar capsule 3·4 μ.

Remarks. This form resembles *Leptotheca latesi* Chakravarty, 1943, in shape of the sporoplasm and polar capsules, and while in size it resembles

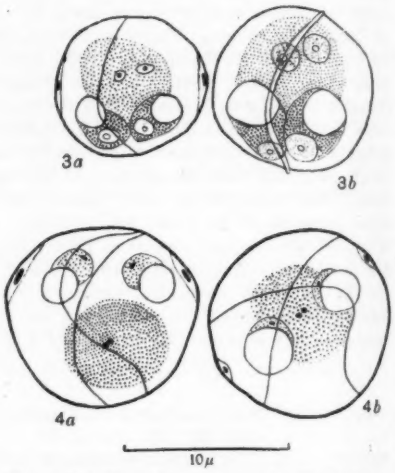

Fig. 3. (*a*) *Sinuolinea murmanica* Basikalowa, 1932, spore. (*b*) *S. cyclopterina* Basik. 1932, spore (both figures redrawn from Basikalowa).

Fig. 4. *Sinuolinea rebae* n.sp. (*a*) spore, front view; (*b*) spore, anterior view.

Leptotheca vikrami n.sp. (Fig. 5 *a–e*)

In the gall bladder of *Zeus faber* both mature and young trophozoites of this species were obtained in abundance. The young trophozoite is spherical in shape with granular protoplasm (11·5 μ in diameter). The mature trophozoite is spherical (34·5μ diameter) and disporous. The protoplasm is not differentiated into ecto- and endoplasm.

The spore is ovoidal in front view, but the posterior margin has slightly less curvature. The sutural line is faint and divides the spore into two unequal shell valves which are thin and smooth. The polar capsules are spherical and each is surrounded by a thin pellicle of the capsulogenous cell the nucleus of which is inconspicuous. The polar filament was not extruded on the application of 5 % KOH. The sporoplasm is granular, having two nuclei. It does not fill the whole of the extracapsular cavity of the spore, but has the form of a triangle the apex of which is directed towards the

L. polymorpha (Thélohan, 1895), it differs from both of them in all other characters.

Zschokkella russelli n.sp. (Fig. 6 *a–d*)

In the gall bladder of *Gaidropsarus tricirratus*, but only rarely in that of *Ciliata mustela*, numerous spores of this species were observed.

The young trophozoite is circular, measuring 72 μ in diameter, very few of which were found. The ectoplasm and endoplasm could be differentiated neither in the young nor in the mature trophozoite. The mature trophozoite of this polysporous form is irregular in shape and contains twenty to forty-five spores in various stages of development. The protoplasm has some refringent granules which disappear in fixed and stained preparations. The mature trophozoite measures up to 238 × 272 μ.

The spore, which has rounded ends, is gibbous in front view and in sutural view is ellipsoidal. The shell valves are thin and decorated with nine to

twelve clear, straight striae. The sutural line is prominent, in the form of an elongated S. The sporoplasm is trapezoidal in shape with concave shorter sides; it is granular, and contains one or two centrally placed nuclei. The polar capsules are spherical and placed at each end of the spore. The coiled polar filament could not be seen within the capsule, but it was extruded when the spore was

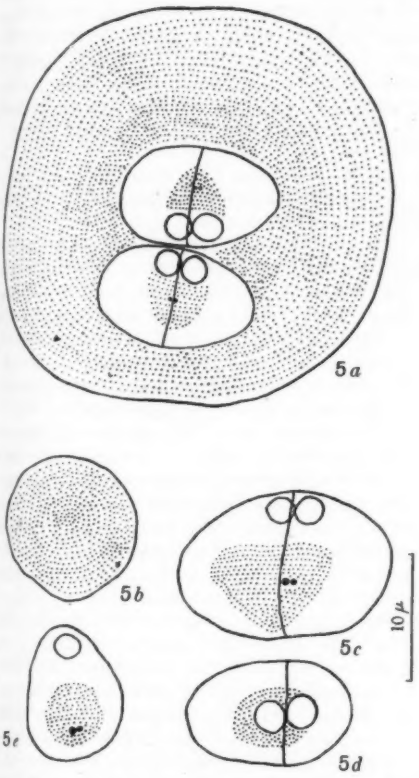

Fig. 5. *Leptotheca vikrami* n.sp. (*a*) young trophozoite; (*b*) mature trophozoite with two spores; (*c*) spore, front view; (*d*) spore, anterior view; (*e*) spore, side view.

treated with 5% KOH. The dehiscence of the polar capsule is directed towards the angles of the spore. The size range of the spore is: length 13·2–16·6 μ, breadth 8·6–9·9 μ, diameter of the polar capsule 2·5–3·5 μ, and length of polar filament 46·6 μ.

Remarks. This form resembles *Zschokkella auerbachi* (Weill, 1929) in shape of the spore only, but differs from it in other respects. Dunkerley (1921) described *Z. ovata* from the gall bladder of *Ciliata mustela* and *Gaidropsarus tricirratus*, but this species differs from the present one in both the shape and the size of the spore, Dunkerley's form being smaller and having smooth shell valves. Moreover, he figured the spores as kidney-shaped, whereas in the present species both sides are convex though with different degrees of convexity. No form resembling that of Dunkerley

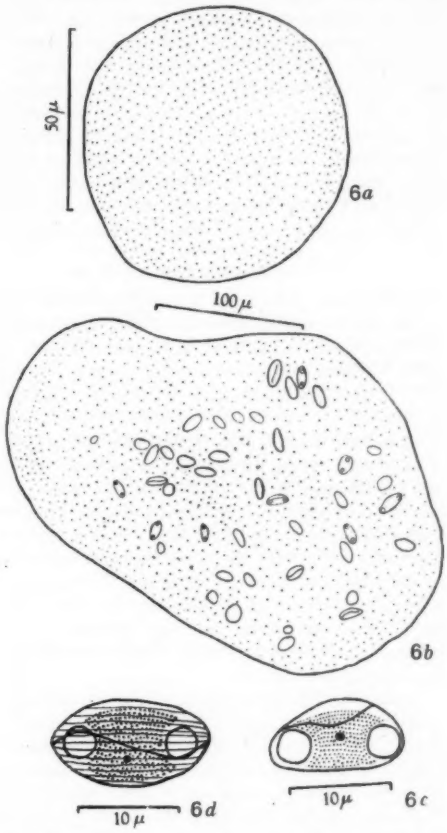

Fig. 6. *Zschokkella russelli* n.sp. (*a*) young trophozoite; (*b*) mature trophozoite; (*c*) spore, front view; (*d*) spore, sutural view.

has been found in these fishes during the present investigations.

Another species of *Zschokkella* was observed as a light infection in the gall bladder of a single specimen of a sturgeon, *Acipenser sturio*, on 19 May 1946, but unfortunately the smears were lost and no permanent preparations could be made. No trophozoites were observed, but a description of the spore from notes made at that time is included here. The

spore was subhemispherical in front view and ellipsoidal in sutural view, the ends were only slightly rounded. The sutural line was in the shape of an elongated S. The shell valves were thin and decorated with eight to ten straight striae. No precise note was made on the shape of the sporoplasm. The polar capsules were spherical and placed near the ends of the spore. The dimensions of the spore were: length 10·8–14·5 μ, breadth 6·8–9·3 μ, and the diameter of polar capsule was 3·5–3·6 μ. Thus it seems to resemble *Zschokkella russelli* n.sp. more than any other species of the genus, yet they are not conspecific, so that it is designated *Z. sturionis* n.sp. As far as the author is aware, this is the first record of a myxosporidian parasitic in Chondrostei. Sachwatkin (1935, 1936) examined fishes of the Kama River Basin for all parasites, but in *Acipenser* spp. he reported no myxosporidians, though he had examined some twenty fishes in all.

SYSTEMATIC DISCUSSION

Zschokkella Auerbach, 1910. There has been a difference of opinion in regard to the generic names *Cystodiscus* Lutz, 1889 and *Zschokkella* Auerbach, 1910. Cordero (1919) made *Cystodiscus* synonymous with *Myxidium* Butschli, 1882 on the basis of the resemblance of the spore characters in these two genera, but R. Weill (1929), probably being ignorant of Cordero's work, immersed the genus *Zschokkella* in *Cystodiscus*. Kudo (1933), on the basis of Cordero's observations, separated *Zschokkella* from *Cystodiscus* and placed the latter under *Myxidium*. Chakravarty (1941) followed this ruling and gave an emended definition of the genus *Zschokkella*. The author is inclined to follow Kudo's view. In this genus there are now fourteen species, of which seven species have the shell valves striated and in seven they are smooth.

Leptotheca Thélohan, 1895. Jameson (1929, p. 60) proposed the merging of *Leptotheca* Thélohan, 1895 with *Ceratomyxa* Thélohan, 1892, because it appeared to him that the only difference between them is in the shape of the spore which varies so widely that the genera overlap. Kudo (1933, p. 195) disagreed with Jameson and re-established *Leptotheca* and *Ceratomyxa*, separating the latter by the breadth of the spore, being more than twice the sutural diameter, whereas in *Leptotheca* the breadth is twice, or less than twice, the sutural diameter. Contrary to the implications of Kudo's definition, the micro-habitats of the species of the two genera are similar. Moreover, *Leptotheca* has come to overlap *Sphaerospora* Thélohan, 1892 (with spherical spores), for example, *Leptotheca spherula* Noble, 1939 has also spherical spores. Yet, while in *Leptotheca* spherical and subspherical spored forms exist alongside species with sausage-shaped spores, the genus *Ceratomyxa* is expressly characterized by the spores being elongated and narrow. Thus, as they stand, the three genera *Sphaerospora*, *Leptotheca* and *Ceratomyxa* form a continuous but overlapping series. In order to remove the ambiguities which have accumulated, it is proposed here to impose upon this group an arbitrary division based on the limiting ratios of length and breadth of the spore. To merge all the three genera into one also becomes impracticable, yet there is no natural segregation. Kudo (1933) did not discuss the relationship between *Leptotheca* and *Sphaerospora*, but subsequent discovery of intermediate forms draws the two genera together, and therefore entirely abolishing the meaning of his suborders. Hence the generic definition must be emended, and *Sphaerospora* removed from its separate suborder Sphaerosporea Kudo, 1920, and included in the family Ceratomyxidae (see p. 116 below).

An emended definition of *Leptotheca* is given here, emphasizing the characters to differentiate it from *Ceratomyxa* and *Sphaerospora*.

Leptotheca Thélohan, 1895, *sensu emend.* Spore ovoidal or arched. Breadth of the spore is twice, or less than twice, but never equal to the sutural diameter. Ends of spore rounded. All occur in the urinary bladder or gall bladder of marine fishes, except two species which occur in the uriniferous tubules of the kidney of frogs. Thirty-one species.

Type species: *L. agilis* Thélohan, 1895.

Sinuolinea Davis, 1917. This genus was created by Davis to include those forms of Sphaerosporidae Davis, 1917 which have a sinuous sutural line. Jameson (1931, p. 65) suggested a rearrangement of the genus: retaining those species which have a 'central chamber' and 'appendages' attached to the shell-valve, under *Sinuolinea* and at the same time removing *Ceratomyxa spinosa* Davis, 1917 to *Sinuolinea*. The rest of the species of *Sinuolinea* he proposed placing in *Sphaerospora*. According to Davis, the chief character of the genus *Sinuolinea* is the spherical spore with a sinuous sutural line, and not the presence of the 'central chamber' with the 'appendages' which Jameson has emphasized. Kudo (1933) has not followed Jameson's view and returned *Ceratomyxa spinosa* to its original genus where, according to the present definitions, it remains. The spore of *Ceratomyxa spinosa* Davis, 1917 is not spherical, which is a necessary character of the members of the family Sphaerosporidae Davis, 1917, and Kudo's view is upheld by the present author. The two species of *Sinuolinea* from U.S.S.R., *S. cyclopterina* Basikalowa, 1932 and *S. murmanica* Basikalowa, 1932, are not listed by Kudo in 1933, and owing to the comparative inaccessibility of the periodical, the figures of the spores of these two species are reproduced here (Fig. 3 *a*, *b*), and the following short descriptions are extracts from a translation of the Russian text:

Sinuolinea murmanica Basikalowa, 1932. From the kidney and the urinary bladder of *Ammodytes tobianus*. The trophozoite is circular, 25–30 μ in diameter, containing two to five spores. The spore is spherical and 12–14 μ in diameter. The infection was 14·1 %.

Sinuolinea cyclopterina Basikalowa, 1932. From the urinary bladder of *Cyclopterus lumpus*. The trophozoite is circular and 50–60 μ in diameter. The spore is spherical with an S-shaped sutural line, and a diameter of 14–16 μ. The infection was 100 %.

The total number of species of *Sinuolinea* is eleven, of which only four species have 'appendages' to the shell valves.

A REVISION OF THE CLASSIFICATION OF THE ORDER MYXOSPORIDIA BUTSCHLI, 1881

The first classification of Myxosporidia was given by Thélohan (1892, 1895) on the basis of the spore characters. It was followed in a modified form by Gurley (1894). Doflein (1899, 1901), and following him Auerbach (1910, 1911), Parisi (1912), and Poche (1913) based their classifications mainly on the characters of the trophozoite. Davis (1917) put forward a new classification based on the spore characters, but divided the suborders on the basis of the micro-habitat of the parasite. This was not accepted by Kudo (1920) who gave another classification, which he modified in 1933. He based his classification, dividing the order Myxosporidia into suborders and families, on the following characters:

(*a*) Suborders separated according to the relation of the sutural axis to the greatest diameter of the spore.

(*b*) For families he used different characters, e.g. (i) habitat (Coelozoic or Histozoic, to distinguish Ceratomyxidae from Wardiidae), (ii) number of polar capsules (Sphaerosporidae, Chloromyxidae and Unicapsulidae), (iii) position of the polar capsules and the presence or absence of the iodinophilous vacuole.

As pointed out by Dunkerley (1925, p. 196) the suborder Sphaerosporea Kudo, 1920 consists of forms which are not necessarily spherical or subspherical, e.g. *Chloromyxum caudatum* Thélohan, 1895, *C. quadratum* Thélohan, 1895, and *C. histolyticum* Perard, 1928. On the other hand, some species of *Leptotheca* have been described which are very nearly spherical, e.g. *L. spherula* Noble, 1939 and *L. abbreviata* Davis, 1917.

An effort has been made here to give a new classification of the order Myxosporidia based on the spore characters. Two quite different types of spores occur in Myxosporidia: (*a*) forms in which the spores have one to four polar capsules together, at or near the anterior end of the spore; (*b*) forms in which the spore has two *widely separated* polar capsules, one at each end. Thus to classify Myxosporidia the position of the polar capsules in the spore is a suitable character for separating suborders. In Unipolaria n.nom. the polar capsules are together at one end, and in Bipolaria n.nom. they are situated at the two opposite ends of the spore.

The forms with an iodinophilous vacuole are present mostly in fresh-water fishes. Thus, it is suggested that the presence of a glycogen-like substance in the vacuole may indicate some fundamental difference in the metabolism connected with a phylogenetic dichotomy; here it is used tentatively as a superfamily criterion.

For the separation of the families it has been decided to use the number of polar capsules. It was hinted by Dunkerley (1925, p. 196) that 'the number of polar capsules is, as a rule, a useful guide in the classification of Myxosporidia'. Ceratomyxidae is by far the largest family, including forms all with two polar capsules, yet comprising the entire range of variation in shape; it is here divided into the new subfamilies: Ceratomyxinae, Sphaerosporinae and Myxosomatinae. The central group Sphaerosporinae, having spherical spores; Myxosomatinae having flattened spores, elongated parallel to the sutural line; and Ceratomyxinae having spores elongated at right angles to the sutural line.

Generic characters include general shape of the spore, shape of the sutural line, and the presence and number of tails to the spore. This leaves for the differentiation of the species characters such as absolute size of the spore and the polar capsule; extent of the sporoplasm; and presence of striae and other emergences on the shell valve. The proposed new classification is given below.

Order MYXOSPORIDIA Butschli, 1881

Suborder *UNIPOLARIA* n.nom.

Myxosporidia in which the spore has one to four polar capsules at or near the anterior end. The shape of the spore is variable. The sporoplasm is with or without an iodinophilous vacuole. Histozoic or Coelozoic.

Superfamily CERATOMYXOIDEA n.superfam.

Unipolaria in which the sporoplasm lacks an iodinophilous vacuole.

Type family CERATOMYXIDAE Doflein, 1899.

Family UNICAPSULIDAE Kudo, 1933, *sensu emend.* Ceratomyxoidea, having a spherical or oval spore with one polar capsule at the anterior end.

Genus *Unicapsula* Davis, 1924. Unicapsulidae, having spherical spore, with asymmetrical spore

valves. Histozoic in marine fishes. Type and only species: *U. muscularis* Davis, 1924.

Genus *Coccomyxa* Léger & Hesse, 1907. Unicapsulidae, having the spore ellipsoidal in 'front' view and circular in 'anterior' view. Coelozoic, in marine fish. Type species: *C. morovi* Léger & Hesse, 1907.

Family CERATOMYXIDAE Doflein, 1899, *sensu emend.* Ceratomyxoidea, in which the spore has two polar capsules at or near the anterior end.

Subfamily Ceratomyxinae n.subfam. Ceratomyxidae, in which the largest diameter of the spore is at right angles to the sutural plane. The ends of the spore are pointed or rounded.

Genus *Ceratomyxa* Thélohan, 1892. Ceratomyxinae, in which the spore is arched, and the breadth is more than twice the sutural diameter. The ends of the spore pointed or rounded. All species in the gall bladder or urinary bladder of marine fishes. Type species: *C. arcuata* Thélohan, 1892.

Genus *Leptotheca* Thélohan, 1895, *sensu emend.* Ceratomyxinae, in which the spore is ovoidal or arched. Breadth of the spore is twice, or less than twice, but never equal to, the sutural diameter. The ends of the spore are rounded. All species in urinary or gall bladder of marine fishes, except two species which occur in the uriniferous tubules of the kidney of frogs. Type species: *L. agilis* Thélohan, 1895.

Genus *Myxoproteus* Doflein, 1898, *sensu emend.* Davis, 1917. Ceratomyxinae, in which the spore is pyramidal, with or without distinct processes from the base of the pyramid. In the urinary bladder of marine fishes. Type species: *M. ambiguus* (Thélohan, 1895) Doflein, 1898.

Genus *Wardia* Kudo, 1920. Ceratomyxinae, in which the spore is isosceles-triangular, with two convex sides and oval in anterior view. Shell valves with ridges or fringe-like posterior processes. The polar capsules are large and spherical, and are near the anterior end of the spore. Histozoic or Coelozoic parasites of fresh-water fishes. Type species: *W. ovinocua* Kudo, 1920.

Genus *Mitraspora* Fujita, 1912, *emend.* Kudo, 1920. Ceratomyxinae, in which the spore is circular or ovoidal in front view, with pyriform polar capsules. The shell valves have fine longitudinal striations with, or without, long posterior filaments. In the kidney of fresh-water fishes. Type species: *M. cyprini* Fujita, 1912.

Subfamily Sphaerosporinae n.subfam. Ceratomyxidae, in which the spore is spherical with two polar capsules, at or near the anterior end.

Genus *Sphaerospora* Thélohan, 1892. Sphaerosporinae, in which the sutural line is straight, and the polar capsules are at the anterior end. Histozoic or Coelozoic parasites of marine or fresh-water fishes. Type species: *S. divergens* Thélohan, 1892.

Genus *Sinuolinea* Davis, 1917. Sphaerosporinae, in which the sutural line is sinuous and the polar capsules are near the anterior end. Shell valves with, or without, appendages. Coelozoic parasites in marine fishes. Type species: *S. dimorpha* (Davis, 1916) Davis, 1917.

Subfamily Myxosomatinae n.subfam. Ceratomyxidae, in which the largest diameter of the spore is along the sutural plane.

Genus *Myxosoma* Thélohan, 1892 (= *Lentospora* Plehn, 1905). Myxosomatinae, in which the spore is ovoidal and flattened with pyriform polar capsules. Histozoic parasites of marine and fresh-water fishes. Type species: *M. dujardini* Thélohan, 1892.

Family TRILOSPORIDAE n.fam. Ceratomyxoidea, in which the spore has three polar capsules at the anterior end.

Genus *Trilospora* Noble, 1939. Trilosporidae, in which the spore is triangular in anterior view, each side of the triangle being concave, and oval in 'front' view. The shell valves are without distinct sutural ridges. In the gall bladder of tide-pool fishes. Type and only species: *T. californica* Noble, 1939.

Family CHLOROMYXIDAE Thélohan, 1892, *sensu emend.* Ceratomyxoidea, in which the spore has four polar capsules.

Genus *Chloromyxum* Mingazzini, 1890. Chloromyxidae, in which the spore is subspherical or ovoidal in shape. Histozoic or Coelozoic parasites of marine and fresh-water fishes, rarely in Amphibia and insects. Type species: *C. leydigi* Mingazzini, 1890.

Genus *Agarella* Dunkerley, 1915. Chloromyxidae, in which the spore is ovoidal and flattened, with long posterior processes. Histozoic parasites of fresh-water fishes. Type and only species: *A. gracilis* Dunkerley, 1915.

Superfamily MYXOBOLOIDEA n.superfam.

Unipolaria, in which an iodinophilous vacuole is present in the sporoplasm. Shape of spore pyriform, ovoidal or pyramidal with one or two polar capsules.

Family MYXOBOLIDAE Thélohan, 1892. Myxoboloidea, in which the spore has two polar capsules at the anterior end. Shape of the spore ovoidal or pyramidal.

Genus *Myxobolus* Butschli, 1882. Myxobolidae, in which the spore is ovoidal and flattened, the shell valves of which lack posterior processes. Histozoic usually in fresh-water fishes. Type species: *M. mülleri* Butschli, 1882.

Genus *Henneguya* Thélohan, 1892. Myxobolidae, in which the spore is ovoidal and flattened, with one or two posterior prolongations ('tails') and pyriform polar capsules. Histozoic, usually in fresh-water fishes. Type species: *H. psorospermica* Thélohan, 1892.

Genus *Hoferellus* Berg, 1898. Myxobolidae, in which the spore is pyramidal with two posterior spinous processes arising from the lateral faces of the shell valve. Parasites in the kidney of fresh-water fishes. Type species: *H. cyprini* (Doflein, 1898) Mercier, 1908.

Family THELOHANELLIDAE n.fam. Myxoboloidea, in which the spore is pyriform and flattened, with one polar capsule at the anterior end.

Genus *Thelohanellus* Kudo, 1933. With the characters of the family. Type species: *T. piriformis* (Thélohan, 1892) Kudo, 1933.

Suborder *BIPOLARIA* n.nom.

Myxosporidia, in which the two polar capsules are widely separated, one at each end of the spore. Sporoplasm is without an iodinophilous vacuole.

Family MYXIDIIDAE Thélohan, 1892. Bipolaria, in which the spore is fusiform, or semicircular, or gibbous in front view.

Genus *Myxidium* Butschli, 1882 (= *Cystodiscus* Lutz, 1889). Myxidiidae, in which the spore is fusiform with pointed ends, having a straight sutural line which coincides with, or makes an acute angle with, the largest diameter of the spore. The polar capsules are pyriform with a long and fine polar filament. Coelozoic or histozoic parasites of marine and fresh-water fishes. Type species: *M. lieberkühni* Butschli, 1882.

Genus *Sphaeromyxa* Thélohan, 1892. Myxidiidae, in which the spore is fusiform with truncate ends. The polar capsules are pyriform with a short and thick polar filament. Coelozoic, in marine fishes. Type species: *S. balbianii* Thélohan, 1892.

Genus *Zschokkella* Auerbach, 1910, *sensu emend.* Chakravarty, 1941. Myxidiidae, in which the spore is semicircular or gibbous in front view, and ellipsoidal in sutural view; with the ends angular or rounded. The sutural line is curved. The polar capsules are large and spherical or pyriform, with a long and fine polar filament. Coelozoic in marine and fresh-water fishes, rarely in Amphibia and reptiles. Type species: *Z. hildae* Auerbach, 1910.

The above classification is based on spore characters only, and may appear somewhat artificial, and for the convenience of identification, little attempt has been made to incorporate speculations on phylogenetic relationships. There is no correlation between the existing systematic groups and their distribution in the lower vertebrates. Thus the monotypic genus *Agarella* is found only in a lungfish (Dipnoi); and the commonest genus in Elasmobranchs, *Chloromyxum*, occurs also in so comparatively recent a teleost as *Scomber scombrus*, as well as in Amphibia. Similar examples could be quoted from other genera. The unicapsulate forms (*Coccomyxa* spp.) were supposed to be intermediate forms between microsporidia and myxosporidia by Léger & Hesse (1907) and Georgevitch (1926), partly owing to the proportionately large polar capsule. Since then, other unicapsulate genera (*Thelohanellus* and *Unicapsula*), have been described, some of which have a relatively smaller polar capsule. At the present time, no useful purpose can be served by speculating as to whether the unicapsulate forms are primitive, and represent a transitional group from Microsporidia, or that the number of polar capsules has been secondarily reduced from more typical myxosporidians. On the other hand, it may be that all these unicapsulate forms do not represent a natural group, and that their origins are various. Further work on comparative study of the life history of the myxosporidians, and other related Protozoa, may in future help towards a more natural classification of this Order.

SUMMARY

Four new species of myxosporidian parasites are described from marine fishes from Plymouth: *Sinuolinea rebae* n.sp., from the urinary bladder of *Solea solea*; *Leptotheca vikrami* n.sp., from the gall bladder of *Zeus faber*; *Zschokkella russelli* n.sp., from the gall bladder of *Gaidropsarus tricirratus* and *Ciliata mustela*; and *Zschokkella sturionis* n.sp., from the gall bladder of *Acipenser sturio*.

The various terms used in describing the spore of Myxosporidia are reviewed and revised.

A new classification is proposed of the order Myxosporidia.

I wish to thank the Director of the Marine Biological Association's Laboratory for all the facilities he has enabled me to enjoy during my stay at Plymouth, by dedicating a new species of *Zschokkella* to him. As a mark of gratitude, and with a deep sense of respect to His Highness the Maharaja of Narsinghgarh State, C.I., I take the liberty of dedicating to him the new species of *Leptotheca* I have discovered. But for his encouragement and help in the early stages of my career it would have been impossible for me to apply for the India State Scholarship which I now hold. I take this opportunity of expressing my thanks to Miss N. G. Sproston for her unfailing help and encouragement during the present studies and am very grateful to her for going through the manuscript critically.

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EXPERIMENTS WITH MICE AND FLEAS

I. THE BABY MOUSE

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(With 2 Figures in the Text)

We need a fuller understanding of the feeding habits of fleas on rodents, both to help in the study of the epidemiology of plague and also in carrying out laboratory experiments on the same subject. In an earlier paper (Buxton, 1938) I described a 'synthetic mouse hole' in which I kept a rodent with a population of fleas (*Xenopsylla cheopis*) under controlled conditions of climate and feeding for periods of many days; with this I obtained some measure of the progeny which resulted under a variety of conditions. There was evidence that the mouse ate a number of the fleas, and I never controlled this variable, which was erratic and sometimes very large. We attempted to avoid the destruction of the fleas by feeding them on a baby mouse, which was changed at intervals, so that there was always one in the experimental vessel. In a postscript to the above paper it was shown that the baby mouse does not consume the fleas, almost all of which are alive when the experiment has lasted a week; in that period the adult mouse may eat about half or more of the fleas. Further experiments are in progress on the factors which determine the destruction of fleas by the mouse, and on the proportion of fleas which are actually found on the mouse under different conditions of temperature and so forth. In the present note, I shall confine myself to studying the baby mouse, as a host of *X. cheopis*. All the experiments here described were carried out between 1937 and 1939, though it has not till now proved possible to tabulate and consider the results. The execution of the work was in the hands of Mr S. Smith, whose help is acknowledged.

The strain of *X. cheopis* was brought to us from Ceylon in 1930 by Dr L. F. Hirst; very much work has been done on it by Leeson, Mellanby, myself and others. The word 'mouse' refers to white or black and white laboratory mice, of no particular strain.

In the paper quoted above it was shown that the proportion of fleas actually on the body of the rodent at the end of the experiment was influenced by temperature, and by the age of the mouse; for instance:

Temp. ° C.	Mouse	Surviving fleas 7th day (originally 400)	% of survivors on mouse
31	Adult	113	21
31	Baby	396	55
25	Adult	206	39
25	Baby	396	30

It is clear that these differences are important, particularly in relation to the 'flea count', so much used by plague workers. As to the biological cause of the differences, we are ignorant. At least they show that baby and adult mice are very different, considered as hosts. The differences might be due to activity or body temperature, and it is to be remembered that the baby mouse has an imperfect control of its temperature.

One may approach the subject by taking a baby mouse from its mother, putting it on the laboratory bench and putting some unfed *X. cheopis* upon it. After 15 min. a considerable proportion of the fleas (35–50 % in a series of experiments) will have fed and one judges by eye that they have taken a full meal. They seem to feed equally well on a very young mouse which is pink and unable to walk, and those which are slightly hairy and in the toddling stage, say 10 days old. One may carry the experiment a little further by taking two babies from the same litter, one straight from the mother, the other kept at about 20° C. or a lower temperature for an hour. If one puts fleas on both, one finds that in some experiments the higher proportion of insects feed on the warm baby, in others the reverse. Simple experiments of this type give no support to the idea that the baby mouse might be a bad host for the flea because it was cold.

One may go further, taking the production of fleas' eggs as a measure of the efficiency of the host. It will be shown later that at 30° C. fleas fed on an adult mouse start laying eggs after a delay of 1 day. The daily production of eggs is not known, but it leads to a production of about seven *adult* offspring per female parent per day. The production of eggs was therefore seven or more.

With baby mice it is easy to count the eggs

produced daily, the mouse being kept on black paper in a small jar. We compared the egg production from baby mice of different ages, a large number of litters of mice being always available. We worked with three groups of fleas, one of which (C and F, Table 1) was fed on a succession of babies always between 2 and 4 days old and weighing 2·0–3·5 g.: these were changed daily for a fortnight so that this group of fleas always had a baby of the same age and weight. Other groups of fleas were fed on middle-sized and larger babies: these last were able to toddle about, so to make for uniformity all baby mice were confined in small cylinders of perforated zinc. The fleas were unfed, under 24 hr. old, and ten males and ten females were put on each mouse. All the work was at 30° C.: two groups of experiments were performed (C, D, E and F, G, H, Table 1).

The same technique was then used to compare egg production of fleas fed on baby mice, some of the fleas having previously been fed on an adult mouse. In one experiment we started with fleas which were unfed and less than 48 hr. old. One lot of fleas (A) were then put on an adult mouse once only for a period of 3 hr. Ten males and ten females which had fed well were then selected, and put on the baby mouse on black paper. A second lot of ten males and ten females (B) were never put on the adult mouse, but straight on a second baby, which was the only mammal on which they ever fed. Baby mice were changed daily: all were 4–5 days old. Both experiments were incubated at 30° C. If one expresses the results in eggs per female per day, one finds that the A fleas laid their first egg on day 4, the B fleas not till day 7. Thereafter the production in the two batches was as shown in Fig. 1. One notes that by comparison with an adult mouse (Table 2, 30° C.) even the A fleas start laying late and never reach a high level of production, particularly if one allows for the fact that the figures in the table are for adult progeny, and those given in Fig. 1 are for eggs. When, however, one compares the A and B fleas it is clear that even a brief, single period of feeding on the adult mouse had a long-lasting effect on the production of eggs: not only did the A fleas start laying eggs earlier but on every day up to the eighteenth their production was higher than that of the B fleas. The mean egg

Table 1. *Groups of* Xenopsylla cheopis *(ten male, ten female, unfed) are fed on a succession of baby mice of different sizes and ages*

The experiments were repeated, C and F being on very small mice, and so forth. 30° C.

	C	D	E	F	G	H
Mouse: Age (days)	2–4	5–8	9–10	2–4	5–8	9–11
Weight (g.)	2·5–3·5	4·0–5·5	4·5–8·0	2·0–3·5	4·0–5·0	5·5–7·5
First egg (day)	4	3	3	3	2	1
Mean production per ♀: 14 days	19·3	35·0	46·0	—	—	—
10 days	—	—	—	9·8	23·2	35·2

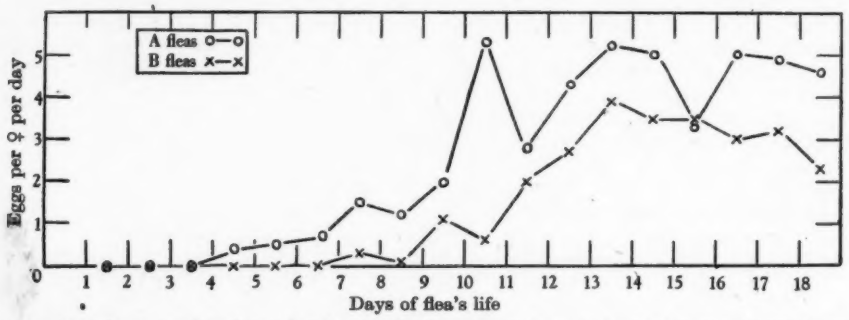

Fig. 1. Number of eggs produced, per female flea (*X. cheopis*) per day: A, fleas fed once only on adult mouse, and then on babies; B, fleas fed only on a succession of baby mice.

The table shows clearly that on the smallest baby (C, F) egg-laying started rather late and the mean number of eggs per female was low compared with the middle-sized mice (D, G): they in turn were less efficient as hosts than the largest young mice (E, H). But even on the largest after egg-laying was established, the daily mean number of eggs per female was only 3·5–5·5, very rarely reaching six. The figure is therefore below that which has already been established for adult mice. A point of some interest is that among the 120 fleas only three deaths occurred in the period of 10–14 days.

production per female, over the 18 days, is forty-five in A, twenty-seven in B.

The above was repeated with a much less clear result. The figures need not be given. The same delay in the starting of egg-laying in B was observed, but after that there was little difference in production; indeed, there were many days on which the two experiments as far as possible identical, the two hosts were each in a sweet jar, a tall glass vessel with an area of 20 sq.in. on the base; the wide mouth was covered with fine wire gauze. The adult mouse received water from above and a daily ration of dry food; the individual mouse was not changed during the experiment. The baby could not be fed

Table 2. *Comparison of* Xenopsylla cheopis *fed on adult and on baby mice*

Bedding and debris incubated daily, and progeny fleas hatched and recorded. Productivity is number of progeny of both sexes per parent female flea per day.

Mouse	Temp. °C.	Fleas	Day of experiment 1	2	3	4	5	6	Total
Adult	22	Parent ♀	25	(25)	(24)	(23)	(23)	22	—
		Progeny ♂	0	0	12	40	67	53	172
		Progeny ♀	0	0	7	35	54	69	165
		Total	0	0	19	75	121	122	337
		Productivity	0	0	0·8	3·3	5·3	5·5	—
Baby	22	Parent ♀	25	25	25	25	25	25	—
		Progeny ♂	0	2	2	5	2	10	21
		Progeny ♀	0	4	1	3	5	8	21
		Total	0	6	3	8	7	18	42
		Productivity	0	0·2	0·1	0·3	0·3	0·7	—
Adult	30	Parent ♀	25	(24)	(23)	(23)	(22)	21	—
		Progeny ♂	0	13	50	?	72	81	?
		Progeny ♀	0	15	48	?	76	75	?
		Total	0	28	98	(153)	148	156	583
		Productivity	0	1·2	4·3	(6·8)	6·7	7·4	—
Baby	30	Parent ♀	24	24	24	24	24	24	—
		Progeny ♂	0	2	6	?	23	27	—
		Progeny ♀	0	0	4	?	17	21	—
		Total	0	2	10	(36)	40	48	136
		Productivity	0	0·1	0·4	1·5	1·7	2·0	—
Adult	32	Parent ♀	25	?	?	?	?	16	—
		Progeny adult	0	0	0	0	7	15	22
		Progeny cocoon	0	1	0	0	17	19	37
Baby	32	Parent ♀	25	25	25	25	25	25	—
		Progeny adult	0	0	0	4	1	2	7
		Progeny cocoon	0	3	3	0	9	7	22
Adult	34	Parent ♀	25	?	?	?	?	11	—
		Progeny	0	0	0	0	0	0	0
Baby	34	Parent ♀	23	23	23	23	23	23	—
		Progeny	0	0	0	0	0	0	0

the B fleas laid more eggs than the A fleas. Further work might perhaps show that the amount of feeding of the A fleas on the adult mouse, or the nutrition of the mothers of the baby mice are critical factors.

A more elaborate experiment was then devised which lasted for 6 days. The purpose was to study the survival of fleas and the production of adult offspring in two vessels, one containing a baby and the other an adult mouse. Care was taken to make but was changed every 24 hr.; it was always found alive after this period. No record was kept of age or weight of baby mice, a serious omission. The bottom of the jar was covered with dry sand; some hay was provided as bedding. In each jar we put twenty-five male and twenty-five female *X. cheopis* unfed and under 24 hr. old. Every day the mice were moved to fresh jars and the living fleas caught up from the bedding and put with the mice. All

the debris* was left in the old jar with any flea eggs there might be, and maintained at 80 % R.H. The emergence of the adult progeny was recorded daily. The jars with the mice and also those undergoing incubation were all kept at 22° C.

This experiment gave several results of interest. The fleas were under 24 hr. old at the start and laid no eggs during the first 2 days in the experimental jars. Afterwards the fleas on the adult mouse laid numbers of eggs which apparently increased daily,

In several respects the experiment was satisfactory. This particular adult mouse refrained from eating the parent fleas, nearly all of which (twenty-five males and twenty-two females) were recovered at the end of the experiment on the sixth day; the figures in brackets (Table 2) indicate the number of females assumed to be alive on intermediate days. There was no mortality among the fleas on the baby mouse. This in itself is evidence that the fleas fed on the baby; had they not done so many fleas

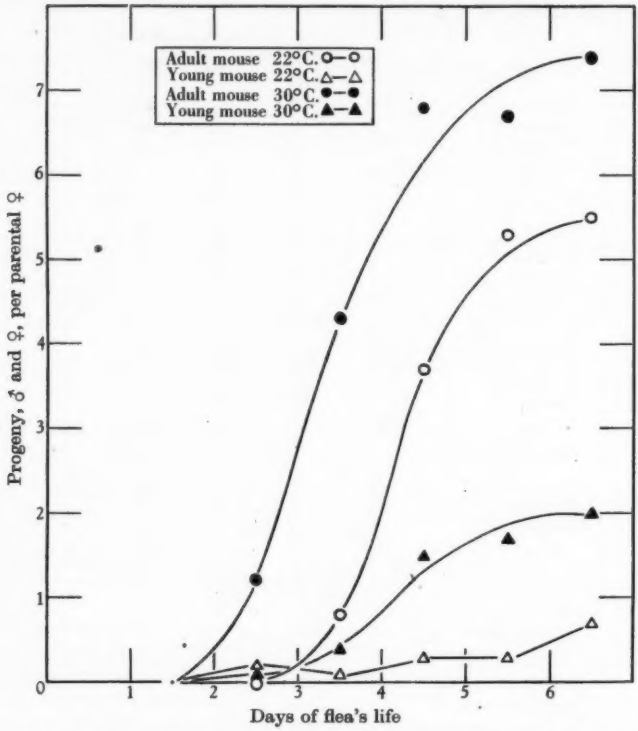

Fig. 2. Production of adult progeny (both sexes) per female parent (*X. cheopis*) per day; fleas are always fed either on baby or adult mice. Two experiments, at 22 and 30° C. Curves are free-hand.

for by about the fifth day one was obtaining more than five adult progeny (male and female) per parent female flea per day. The parent fleas kept on the baby mouse only produced an eighth or tenth of the progeny which came from the fleas on the adult mouse (Fig. 2).

* If I were repeating the work I should give the adult mouse water but no food, so as to eliminate mouse faeces, which may be an important variable in the larval environment. Alternatively, one might add mouse excrement to each jar.

would have been dead by the end of the experiment (see Leeson, 1932, Table 1). In an experiment of this type one does not know how many eggs were laid or anything about mortality in the egg or larval stage; but we know that there was practically no mortality in the cocoons, for 226 were recovered by sieving; of these all but one had hatched satisfactorily; in the one exception there was a dead pupa.

In carrying out this experiment the emergence of the adult progeny from the bedding of each day was recorded. The figures reveal another matter

interest, for one finds that progeny from parents fed on the adult mouse emerge earlier than those from the baby mouse (see Table 3; the two sexes of fleas

Table 3. *Comparison of* Xenopsylla cheopis *fed on adult and baby mice*

Figures give the number of emergences in incubated bedding, from date of oviposition to that of emergence of progeny, 22° C.

	Male fleas		Female fleas	
Days	Adult mouse	Baby mouse	Adult mouse	Baby mouse
33	—	—	9	—
34	—	—	8	—
35	—	—	32	—
36	—	—	29	—
37	—	—	59	3
38	3	—	15	2
39	1	—	10	5
40	6	—	1	7
41	22	—	2	4
42	34	—	—	—
43	45	2	—	—
44	31	4	—	—
45	17	4	—	—
46	7	6	—	—
47	6	3	—	—
48	—	1	—	—
49	—	1	—	—
Total	172	21	165	21

are shown separately because, as is well known, the female of this insect passes through the early stages more speedily than the male). This delay may be due to malnutrition of larvae in the jar fed on baby mice: the fact that fleas reared from those fed on baby mouse show no abnormal 'lagging' in emergence is rather opposed to the suggestion that there had been larval malnutrition. It is already known (Sharif, 1937) that flea larvae require substances which may be supplied by yeast. Under my experimental conditions, there were no mouse droppings in the baby mouse jar: perhaps the droppings may be a source of yeast or other micro-organisms, and in that respect needed by the flea larvae.

The whole experiment was repeated in all respects, except that the jars with the mice were kept at 30° C. and the subsequent incubation of the bedding was carried out at that temperature and 80% humidity. The results (Table 2) are similar in most respects to those obtained at 22° C., the higher temperature naturally gives rise to egg-laying at an earlier day and produces a larger number of progeny per day. The contrast between the adult and the baby mouse is again great; the essential facts are shown in Fig. 2. In this experiment one also finds that the progeny of fleas fed on the adult mouse passed through the early stages more quickly than those from the baby; it does not seem necessary to publish these figures. Owing to an accident to a thermostat the bedding of day 4 was overheated: the cocoons were recovered and the numbers are shown in brackets; all of them contained dead pupae.

Similar experiments were carried out at 32 and 34° C. (Table 2). At both these temperatures the parent fleas survived much better on the baby than on the adult mouse. Other unpublished facts show that the adult mouse consistently kills more fleas at higher temperatures, presumably because the fleas are more active and irritating. It is of some interest that the fleas live successfully at least for 6 days even at so high a temperature as 34° C. As to the progeny at 32° C. a considerable number of cocoons were formed; in these the adult hatched from the pupa but failed to escape from the cocoon. Even if one adds the figures for the cocoons to those for fleas actually emerged, the productivity is very low both on the adult and baby mouse, and all the fleas which were bred were exceedingly small. At 34° C. there was no progeny either of adults or cocoons. As the experimental jars were moist owing to the presence of the mouse and as the bedding was subsequently incubated at a high humidity, it is probable that these results are due to the high temperature itself and not to desiccation of the larvae.

It is not proposed to discuss these facts at length. They show clearly that as far as life is concerned the baby mouse is an excellent host for *X. cheopis*. For instance, there is almost no mortality among groups of fleas kept for as long as 14 days on babies of various ages at 30° C.; even mice as old as 9–10 days do not catch and eat fleas.

But it is equally clear that fleas fed on the baby mouse tend to produce an abnormally low number of eggs per day and to start laying after an unusual interval. One may contrast the fleas fed on an adult mouse at 30° C. (Fig. 2 and Table 2) which produce an *adult* progeny of about 7 with those fed on baby mice even of 9–10 days which generally lay only 3·5–5·5 *eggs* at the same temperature. It is clear also that whatever the deficiency may be, it is greater the smaller the mouse. This comes out very clearly in both groups of figures in Table 1.

It is also remarkable that the deficiency may be overcome, at least to some extent, if the fleas are first allowed to feed for a few hours on an adult mouse; thereafter, if fed on nothing but baby mice they continue for at least 18 days to lay more eggs than if they had not had that single feed on the adult mouse.

The experiments clearly demonstrate that fleas fed on a baby mouse lack something: they start to lay eggs after an unusual delay, and produce

less than the normal number of eggs. The deficiency might be in nutrition: against this is the fact that these fleas feed well and do not tend to die abnormally early. It is more probable that they lack a mammalian sex hormone, which they would normally derive from the blood of an adult rodent: there is slight evidence in the literature supporting the view that a mammalian hormone may have this effect on a blood-sucking Arthropod. I propose to examine this question in the near future.

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DRUG-RESISTANCE IN *PLASMODIUM GALLINACEUM*, AND THE PERSISTENCE OF PALUDRINE-RESISTANCE AFTER MOSQUITO TRANSMISSION

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CONTENTS

	PAGE
I. Introduction	125
II. Methods	126
III. Attempts to increase the natural resistance of *Plasmodium gallinaceum* to mepacrine hydrochloride	127
IV. The effect of continued treatment with pamaquin upon *P. gallinaceum*	127
V. The effect of continued treatment with paludrine HCl upon *P. gallinaceum*	128
VI. The development of drug-resistant exo-erythrocytic parasites in the paludrine-resistant strain of *P. gallinaceum*	130
VII. The effect of mepacrine, pamaquin and M. 4430 upon the paludrine-resistant strain of *P. gallinaceum*	130
VIII. The immunological relationship of the paludrine-resistant and normal strains of *P. gallinaceum*	132
IX. The effect of mosquito-transmission upon paludrine-resistance in *P. gallinaceum*	132
X. Discussion	134
XI. Summary	136
References	137

I. INTRODUCTION

Although drug-resistance has frequently been described in bacteria and trypanosomes there are few references to it in the literature upon experimental malaria. In one canary amongst many infected with *Plasmodium relictum* and treated with quinine, the Sergents (1921*a*, *b*) describe the multiplication of parasites in spite of continued treatment with the drug in doses which, during the preceding 9 months, had controlled the infection. This resistance to quinine was preserved in two further passages through canaries by blood inoculation, but was partially lost in the third passage. Kritschewski & Rubinstein (1932) compared the incubation period, in *Acanthis linaria*, of two strains of *Plasmodium relictum* treated with quinine for 6 successive days following the day of inoculation, one strain having received 4–6 similar courses of quinine previously in as many successive passages, whereas the other had received no previous drug treatment. In 78·3 % of the birds infected with the quininized strain parasites appeared in the peripheral blood within 4 days of the cessation of treatment, whereas no parasites were seen within the same period in the birds infected with the normal strain. The increase in resistance claimed was thus very slight. Kritschewski & Halperin (1933) found that sometimes, though not invariably, this quinine-resistance persisted after the strain had undergone cyclical development in *Culex pipiens*. No resistance to plasmoquine was observed in a strain treated similarly with that drug. Lourie (1935) subjected *Plasmodium cathemerium* in canaries to a much more prolonged and rigorous treatment with quinine and plasmoquine, but failed to obtain any evidence of resistance to either compound. He points out that during a prolonged period of drug treatment habituation of the host to the drug may arise, involving a greater destruction of the drug in the tissues, or a failure to absorb it readily. In order to avoid these contingencies he subjected *P. cathemerium* to prolonged periods of drug treatment by passage at frequent intervals through a series of birds undergoing treatment with quinine or plasmoquine, but no resistance to either drug developed.

Fulton (1942) made thirty-three passages, in 15½ months, by blood-inoculation through fowls receiving 2 mg./kg. of plasmoquine in the earlier passages, up to 20–30 mg./kg. in the later experiments, but no resistance to the drug was acquired. A shorter and more intensive course of treatment with the same compound also failed to produce resistance. Fulton observed exo-erythrocytic parasites in the internal organs of a large proportion of the fowls undergoing treatment with plasmoquine.

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He points out that these exo-erythrocytic parasites are believed to be insusceptible to the action of the drug, and Coulston & Manwell (1940, 1941) have shown that they can arise from erythrocytic forms. Fulton therefore suggests that failure to induce an increased resistance to plasmoquine in *P. gallinaceum* may be due to the formation, during drug-treatment, of a reservoir of exo-erythrocytic parasites unaffected by the drug, and that these may give rise later to fresh infections of erythrocytic parasites also unaffected by the drug.

Nauck (1934) claimed to have induced resistance to quinine and plasmoquine in *P. knowlesi* in *Macacus rhesus* and *M. cynomolgus*, and Fulton & Yorke (1941) were able to increase the resistance of this parasite to plasmoquine up to the maximum dose which the host would tolerate. Whereas in the early passages a course of four doses, each of 3·3 mg./kg., of plasmoquine given intramuscularly, caused a temporary disappearance of the parasites, in later passages four doses, each of 13·2 mg./kg. (M.T.D.), had no effect. A second strain of *Plasmodium knowlesi* resistant to plasmoquine was later prepared (Fulton & Yorke, 1943), and maintained without further drug-treatment, in a state of chronic infection for 5 months. When inoculated into a fresh host it still showed an enhanced resistance to the drug.

If Fulton's failure to enhance resistance to plasmoquine in *P. gallinaceum* were due to the formation of a reserve of exo-erythrocytic parasites unaffected by the drug, the experimental methods used by him could not lead to an increase in resistance to any drug devoid of action upon exo-erythrocytic parasites. In his experiments a total of thirty-three passages was made in 15½ months, which means that the infection must, on an average, have been allowed to run for up to a fortnight in each bird before it was transferred to a new host. Such a period would permit the development of exo-erythrocytic forms. It seemed to us possible that if the conditions of the experiment were such that the development of the insusceptible exo-erythrocytic forms was kept to a minimum, and the susceptible erythrocytic forms were constantly present in large numbers, the resistance of the parasites to a drug might be increased above the normal by continuous treatment. Using a technique designed to produce such a condition we attempted to enhance the natural resistance of *P. gallinaceum* to mepacrine, pamaquin and paludrine.

II. METHODS

In experiments to be described elsewhere we have found it possible to passage a strain of *P. gallinaceum* in a patent state by intravenous inoculation made daily, or every second day, into chicks 45–65 mg. in weight. Provided that the inocula are sufficiently large, and that the initial inoculum is taken from a donor with a heavy infection, it is possible to ensure that the infections resulting in each bird within the 24 or 48 hr. between inoculation and the withdrawal of blood will be heavy. Our practice was to inoculate into the smaller jugular vein on the left-hand side of the neck, and to withdraw blood from the larger vein on the right-hand side. If the same vein is used twice within a short period of time haematomata produced at the first operation may render the second difficult.

In the earlier experiments attempting to induce drug-resistance inocula of 0·5–1·0 c.c. of parasitized blood were used, and the inoculations were made daily or every second day. Later, particularly when some degree of resistance to a drug had been acquired, the volume of blood was reduced to 0·25 c.c. or less, and the passages were made at intervals of 2–4 days. The blood was mixed with a little heparin in 0·85 % NaCl solution to prevent coagulation.

In post-mortem examinations of birds inoculated intravenously with a strain of parasite which had been subjected to a series of these rapid, intravenous passages, exo-erythrocytic parasites were rarely found before the 5th or 6th days. The number of erythrocytic parasites arising from them in the first 24–48 hr. after inoculation must therefore be negligible in comparison with the total population of parasites.

The compounds used in the following experiments were: pamaquin (plasmoquine) N-diethylamino-isopentyl-8-amino-6-methoxyquinoline; mepacrine hydrochloride (atebrin) 2-methoxy-6-chlor-9-*N*-diethylamino-isopentylaminoacridine; paludrine hydrochloride N^1-*p*-chlorophenyl-N^5-isopropylbiguanide. The drugs were administered orally in aqueous solution through a piece of catheter tube on the end of a graduated syringe. The doses were adjusted to the body weight of the bird and calculated in mg./20 g. Birds varying from 40 to 60 g. were normally used. Each bird received one dose of drug on the day preceding inoculation, and either one or two doses on the day of inoculation and on each succeeding day.

From time to time during the course of the experiments tests were made in order to discover whether the resistance of the parasites to a drug had increased. These tests were made by a modification of the method described by Curd, Davey & Rose (1945*a*) for the testing of anti-malarial compounds. Blood, withdrawn from a chick infected with the strain of parasite to be tested for drug-resistance, was mixed with sufficient heparin-Ringer solution to produce a dilution of 50,000,000 parasites/0·25 c.c. of the mixture. Groups of chicks were inoculated intravenously with approximately 50,000,000 parasites per chick. One-half of the group was treated

with the drug under test, and the other half remained untreated as controls. A similar group of chicks was inoculated intravenously with the same number of parasites of a strain of *P. gallinaceum* which had been passaged every 2–3 days through normal chicks, but which had never been treated with a drug. This strain was therefore strictly comparable with the drugged strains except for the omission of drug treatment. One-half of the group was treated with similar doses of drug to those given to the drugged strain, and the other half remained untreated as controls. One dose of drug was given immediately prior to inoculation and one or two doses on each of the following 3 days. Blood smears were made on the fourth morning following inoculation, and the number of parasites per 500 corpuscles assessed. A comparison was then made of the average counts for all four groups of chicks.

III. ATTEMPTS TO INCREASE THE NATURAL RESISTANCE OF *PLASMODIUM GALLINACEUM* TO MEPACRINE HYDROCHLORIDE

In attempting to increase the natural resistance of *Plasmodium gallinaceum* to drugs it is necessary, if the effect of the exo-erythrocytic cycle is to be avoided by frequent passage of the strain, that the host should be treated with the drug in doses which will not inhibit the multiplication of the erythrocytic parasites. Whereas a given dose of drug may appear to be without effect when given over a short period, prolonged administration may produce a gradual decrease in the vitality and rate of reproduction of the parasite, leading to a falling off in the intensity of infection. Under such conditions it is impossible to keep the strain in a patent state when passaged at 24–48 hr. intervals. The initial dosage, therefore, should be the largest amount of drug which is without effect upon the erythrocytic parasites when given over a long period. The largest dose of mepacrine HCl which fulfils these conditions is 0·32 mg./20 g. once daily. After the strain had been passaged at 24 or 48 hr. intervals for 17 days through birds receiving this dose, without any visible effect upon the parasites, an attempt was made to increase it to 0·64 mg. once daily. Within 24 hr. the parasites became abnormal, there was a rapid decrease in numbers, and, although inocula of 0·5–1·0 c.c. were used, the infection disappeared after two further passages through birds receiving the higher dose. After a further 24 days' treatment of the strain with doses of 0·32 mg. of mepacrine another similar attempt was made to increase the dose, and although at first many of the parasites showed vacuolation and a large proportion died, there was, in later passages, a gradual increase in number of parasites which were normal in appearance. The occasional passage of the strain for 24 to 48 hr. through birds receiving no drug treatment improved the intensity of infection. After 5 months' passage at 24 or 48 hr. intervals through birds receiving 0·64 mg./20 g. of mepacrine a day the reaction of the strain to this dose was compared with that of the normal strain, by the method described above (p. 126). No significant difference in sensitivity to the action of the drug was observed in the two strains (Table 1).

Table 1. *Comparison of the reaction of the mepacrine-treated and normal strains of* Plasmodium gallinaceum *to mepacrine HCl after 5 months' treatment*

Dose once daily	Normal strain	Drugged strain
0·64	(6) 22	(8) 42

The intensity of infection is expressed as the average number of parasites per 500 erythrocytes in a group of birds. The number of birds in each group is given in parentheses. The dose is, throughout these tables, calculated in mg./20 g. of body weight of bird.

Attempts made at frequent intervals to increase the dose to 0·64 mg. of mepacrine twice daily, or to 1·28 mg. once daily, failed. In each case the parasites became abnormal within 48 hr., and the infection disappeared after one or two passages. It is to be concluded therefore that no significant resistance to mepacrine was induced as a result of over 6 months' treatment (Bishop & Birkett, 1947). The attempt to induce resistance to this drug was therefore discontinued. These results are in agreement with those of Williamson, Bertram & Lourie (1947) who also failed to produce resistance to mepacrine in *P. gallinaceum*.

IV. THE EFFECT OF CONTINUED TREATMENT WITH PAMAQUIN UPON *PLASMODIUM GALLINACEUM*

In May 1946 a strain of *Plasmodium gallinaceum* was inoculated into chicks treated with one dose daily of 0·01 mg./20 g. of pamaquin, which was the highest dose which was without effect upon the blood infection when given over a long period. The strain was passaged at intervals of 24–48 hr., inocula of 0·8–1·0 c.c. of parasitized blood being used. After 6 weeks, as no decrease in intensity of infection had been observed, the daily dose of drug was increased to 0·02 mg./20 g., and a further increase to 0·04 mg. was made 3 weeks later. With the higher dose the parasites showed vacuolation, and became reduced in numbers, but after a few days' treatment their appearance became normal. After sixty-two passages, covering a period of 73 days, through birds receiving 0·04 mg./20 g. of pamaquin per day,

the sensitivity of this strain was compared with that of the normal untreated strain, but no significant difference was seen (Table 2). It was obvious from

Table 2. *Comparison of the reaction of pamaquin-treated and normal strains of* Plasmodium gallinaceum *to pamaquin*

Dose once daily	Untreated birds		Drugged birds	
	Normal strain	Pamaquin strain	Normal strain	Pamaquin strain
0·04	—	—	(8) 32	(7) 19
0·08	(5) 1280	(2) 1233	(6) 9	(3) 346
0·08	(6) 1501	(6) 1204	(6) 1	(6) 587

The intensity of infection is expressed as the average number of parasites per 500 erythrocytes in a group of birds. The number of birds in each group is given in parentheses.

this experiment, in which relatively small inocula (50,000,000 parasites) were used, that the actual rate of growth of the parasites in birds receiving the drug must be very poor and the mortality high. The heavy infections obtained in the serial passages through drugged birds must therefore be due to the large inocula, which disguised the heavy mortality rate.

After a few passages through birds receiving 0·04 mg. of pamaquin a day, and as soon as the parasites became normal in appearance, blood was inoculated into a bird receiving 0·08 mg. of drug daily. Within 24 hr. the majority of the parasites became vacuolated and showed signs of degeneration, but a few remained unaffected by the drug. The strain was passaged at 48 hr. intervals through birds receiving this increased dose, but although inocula of 1·0 c.c. of parasitized blood were used the intensity of infection was very poor. It was found necessary from time to time to pass the strain for 48–72 hr. through a bird receiving no drug in order to keep it in a patent state. Gradually, however, the proportion of parasites affected by the drug became less and the intensity of infection increased, so that after 3 months it was possible gradually to decrease the size of the inoculum. Finally good infections were produced in 48 hr. with 0·1–0·2 c.c. of parasitized blood. It was observed, however, that from time to time the infection produced in individual birds was lighter than in the majority. This was probably due to a greater absorption of drug in the individual bird, resulting in a higher concentration of drug in contact with the parasites.

After 5½ months' treatment with daily doses of 0·08 mg. of pamaquin the sensitivity of the strain to this dose was compared with that of the normal strain (Table 2). The strain which had been exposed to the drug was found to be definitely less sensitive to its action than the normal strain. A resistance to pamaquin had therefore been developed as a result of prolonged treatment with the drug.

Attempts are being made to increase the resistance to pamaquin further by subjecting the strain to two doses daily, each of 0·08 mg./20 g., but up to now the proportion of parasites affected by the drug in each passage is high, and the intensity of infection correspondingly low.

V. THE EFFECT OF CONTINUED TREATMENT WITH PALUDRINE HCl UPON *PLASMODIUM GALLINACEUM*

Davey (1946*a*) has shown that the minimum dose of paludrine acetate effective upon *Plasmodium gallinaceum* is 0·1 mg./20 g. when given twice daily over a period of 3½ days. With this dose the average number of infected corpuscles per 500, in a group of chicks, was 1 as compared with 398 in untreated birds. In our initial attempts to induce resistance to paludrine HCl we decided to use a dose of 0·025 mg. once daily. In the first few passages made at 48 hr. intervals and treated with this dose many of the parasites became abnormal, with nuclei staining faintly, and some showing signs of disintegration; but the infection remained patent, and with each successive subinoculation the proportion of individuals affected by the drug decreased, until finally only normal parasites were seen. Thereafter the passages were made at intervals of 48–72 hr. After fifteen passages covering a period of 29 days the dose was increased to 0·05 mg. once daily (Table 3),

Table 3. *The development of resistance to paludrine HCl in* Plasmodium gallinaceum

Dose per day*	No. of serial passages	No. of days
1 × 0·025	15	29
1 × 0·05	5	11
2 × 0·05	3	8
1 × 0·1	2	6
2 × 0·1	4	9
1 × 0·2	5	11
2 × 0·2	3	8
1 × 0·4	2	3
2 × 0·4	5	18
1 × 1·0	9	18
2 × 1·0	—	—

* In mg./20 g. body weight of bird.

without any apparent effect upon the parasites. Further increases up to 0·4 mg./20 g. twice daily were gradually made without any pronounced effect upon the appearance of the majority of the parasites or upon the intensity of infection, though after each increase in dosage a proportion of the parasites was affected by the drug.

When the dosage had reached 0·4 mg. twice daily two strains were formed:

Strain A was passaged by inocula of 0·1–0·2 c.c. of infected blood through birds receiving 0·4 mg. of paludrine twice daily, and no attempt was made to increase the resistance further.

Strain B was passaged at similar intervals to Strain A but through birds receiving one dose daily of 1·0 mg. of paludrine. At first this dose had a marked effect upon both the appearance of the parasites and the intensity of infection, but gradually both returned to normal. After 18 days' treatment (Table 3) the dose was increased to 1·0 mg./20 g. twice daily, which again led to a temporary reduction in the intensity of infection, though few of the parasites were affected visibly by the drug. Ultimately heavy infections of parasites normal in appearance were obtained in birds receiving this dosage of drug.

After 3 days' treatment with daily doses of 0·4 mg. of paludrine, and a further 10 days' with this dose twice daily, the sensitivity of the strain to the twice-daily dose was compared with that of the normal strain (Table 4). The development of the normal strain, which produced heavy infections in untreated birds, was inhibited completely by the drug, whereas the paludrine-resistant strain produced good infections though not so heavy as in the former experiments, in birds treated with the drug (Table 4).

After passage for a further 6 weeks through birds receiving two doses daily of 0·4 mg. of paludrine the sensitivity of the strain (Strain A) to this dose of drug was again tested. Heavy infections developed in birds inoculated with the paludrine-resistant

Table 4. *Comparison of the reaction of paludrine-resistant and normal strains of* Plasmodium gallinaceum *to paludrine HCl*

		Untreated birds		Drugged birds	
Strain	Dose twice daily	Normal strain	Paludrine-resistant strain	Normal strain	Paludrine-resistant strain
A	0·1			(7) 1	(6) 712
A	0·2			(6) 1	(5) 708
A	0·4	(2) 814		(6) 0	(8) 355
A	0·4	(6) 1001	(6) 1029	(6) 0	(5) 765
B	1·0	(5) 400	(3) 265	(5) 0	(4) 36
B	1·0	(6) 1150	(5) 398	(6) 0	(5) 67
B	0·8				(5) 217
B	0·6				(6) 225

The intensity of infection is expressed as the average number of parasites per 500 erythrocytes in a group of birds. The number of birds in each group is given in parentheses.

When doses of paludrine greater than 1·0 mg. were given twice daily to chicks the proportion which died on the 2nd and 3rd day of treatment was high. No attempt therefore was made to increase the resistance of *P. gallinaceum* to doses greater than 1·0 mg., as with the larger doses there is a danger of losing the strain of parasite by the death of the host unless relatively large numbers of chicks can be used, which was not practicable in our experiments.

After the first 3 months' treatment of *P. gallinaceum* with paludrine in doses increasing from 0·025 to 0·2 mg. the sensitivity of this drugged strain to 0·1 and 0·2 mg. paludrine twice daily was compared with that of the normal strain by the method described on p. 126. In these early experiments no comparison was made of the infections produced by the two strains in untreated birds. Whereas the development of the normal strain was inhibited almost completely (Table 4) by these doses, the drugged strain produced heavy infections in both groups of birds treated with these doses of strain whether treated with paludrine or not (Table 4), whereas infections of the normal strain failed to develop in birds undergoing drug treatment though they were heavy in untreated birds.

The sensitivity of Strain B to twice daily doses of 1·0 mg. of paludrine was tested after it had been passaged for 3 weeks through birds receiving this dose of drug (Table 4). The infections produced in birds treated with the drug were of a very low grade, which suggested that the strain was, as yet, but poorly adjusted to this higher dose of drug. In the untreated control chicks, also, the infections produced by this strain were not heavy, which suggested that the vitality of the strain had been depressed by continual treatment with the large doses of drug.

After 6 weeks' further treatment with doses of 1·0 mg. of paludrine twice daily, the sensitivity of Strain B to doses of 1·0, 0·8 and 0·6 mg. of the drug twice daily was tested (Table 4). Whereas the infections produced in birds treated with 1·0 mg. doses were not heavy, they were of greater intensity in

birds treated with the lower doses. The parasites were all normal in appearance. It is to be concluded, therefore, as stated in a preliminary account of these experiments (Bishop & Birkett, 1947), that a definite resistance to these doses of paludrine had developed. Thus, as a result of continuous treatment with the drug, *P. gallinaceum* was able to tolerate paludrine in doses forty times as great as those which, in the initial passages, had affected the majority of parasites, and ten times greater than the minimum effective dose as determined by Davey (1946*a*). A 20- to 40-fold increase in resistance to paludrine has been obtained independently by Williamson, Bertram & Lourie (1947).

VI. THE DEVELOPMENT OF DRUG-RESISTANT EXO-ERYTHROCYTIC PARASITES IN THE PALUDRINE-RESISTANT STRAIN OF *PLASMODIUM GALLINACEUM*

As the technique of rapid intravenous passage employed in producing the paludrine-resistant strain was designed to limit the development of exo-erythrocytic parasites to a minimum, it is to be concluded that the mechanism by which resistance to the drug is acquired must be one which operates upon the erythrocytic parasites. The question therefore arises whether exo-erythrocytic parasites are formed in a paludrine-resistant strain, and if so, whether the erythrocytic parasites arising from them retain this resistance.

It was observed that in birds inoculated with Strain B, resistant to 1·0 mg./20 g. of paludrine twice daily, and treated for 8 or 9 days with this dosage of drug, exo-erythrocytic parasites appeared in the spleen and liver, though the birds were still undergoing treatment with the drug at the time when they were sacrificed. It can be concluded, therefore, that the administration of paludrine in large doses does not prevent the formation of exo-erythrocytic parasites in birds inoculated with the paludrine-resistant strain of *P. gallinaceum*.

In trying to determine whether erythrocytic parasites arising from these exo-erythrocytic forms retained their resistance to the drug, difficulty was experienced in obtaining an inoculum of exo-erythrocytic parasites absolutely free from erythrocytic forms. As mepacrine is known to eradicate erythrocytic parasites of *P. gallinaceum* and to be without effect upon exo-erythrocytic forms, it was decided to use this drug to free a bird of erythrocytic parasites, whilst retaining the exo-erythrocytic infection. A bird which had been inoculated intravenously with 50,000,000 parasites of the strain resistant to 0·2 mg. of paludrine was treated with similar doses of the drug for 3½ days. An infection of 850 parasites per 500 corpuscles was produced on the 4th day. The parasites were therefore definitely resistant to the drug. The bird was then treated with the maximum dose of mepacrine which the chicks would tolerate (5·12 mg./20 g.) for 7 days. Within 3 days the parasites had disintegrated and disappeared from the peripheral blood. On the 5th day of mepacrine treatment a few small uninucleate parasites appeared in the red blood corpuscles and became numerous on the 7th day. The bird was killed and many exo-erythrocytic parasites were found in its spleen. The spleen was pulped, and a suspension in Ringer's solution was inoculated intramuscularly into two chicks. When parasites became numerous in the peripheral blood of the recipients, doses of 0·2 mg. of paludrine were given twice daily, but heavy infections developed in spite of continued treatment with the drug. None of the parasites appeared to be affected by the paludrine.

Although there is no definite proof that all the erythrocytic parasites in the donor bird had been destroyed by the mepacrine, and that all the parasites injected into the recipients were either exo-erythrocytic forms or uninucleate merozoites derived immediately from them, the greater part of the inocula was known to be composed of exo-erythrocytic forms, or of uninucleate parasites which appeared in the peripheral blood after the destruction of the original erythrocytic infection and at a time when exo-erythrocytic parasites were numerous. It is therefore justifiable to assume that the majority of parasites constituting the infections in the recipients were exo-erythrocytic in origin. As none of the parasites present in the peripheral blood of the recipients appeared to be affected by paludrine it can be concluded that whatever their origin, whether from exo-erythrocytic parasites or from any erythrocytic parasites which might have withstood the action of the mepacrine, they were resistant to the drug. This experiment therefore proves that the exo-erythrocytic parasites formed by a paludrine-resistant strain are themselves resistant to the drug, and that the erythrocytic parasites derived from them retain this resistance.

VII. THE EFFECT OF MEPACRINE, PAMAQUIN AND M. 4430 UPON THE PALUDRINE-RESISTANT STRAIN OF *PLASMODIUM GALLINACEUM*

Since, by continuous treatment with the drug, it is possible to increase the resistance of *Plasmodium gallinaceum* from its low, inherent level up to the maximum dose which the host will tolerate, it was important to determine whether this enhanced resistance is specific to paludrine, or whether it embraces all compounds effective upon *P. gallinaceum*.

In determining whether the paludrine-resistant strains of *P. gallinaceum* had also developed a resistance to mepacrine the dose of that drug chosen was the lowest which had an antiplasmodial effect upon the normal strain of parasite. A dose of 0·64 mg./20 g. of mepacrine, when given once daily for 4 consecutive days beginning with the day of inoculation, produced a decrease in intensity of infection, though the effect varied in degree in individual birds. Doses below this had no effect upon the parasites (p. 127). Even a slight increase in resistance to mepacrine should therefore be detected with this dose of the drug.

the drug was observed between individual birds in both the normal and paludrine-resistant strains, but the majority of parasites present in the blood at the end of treatment, in both strains, was affected by the drug. It can be concluded that the development of resistance to paludrine does not confer a resistance to mepacrine. Williamson, Bertram & Lourie (1947) have also found independently that a strain of *P. gallinaceum* resistant to paludrine retains its normal sensitivity to mepacrine.

The effect of pamaquin (0·08 mg./20 g.) upon the paludrine-resistant strain (Strain B, resistant to 1·0 mg. of the drug) and the normal, untreated strain

Table 5. *Comparison of the reaction of paludrine-resistant and normal strains of* Plasmodium gallinaceum *to mepacrine, pamaquin and M.* 4430

	Untreated birds		Drugged birds	
No. of exp.	Normal strain	Paludrine-resistant strain	Normal strain	Paludrine-resistant strain
		Strain A		Strain A
			Mepacrine 0·64 mg.	Mepacrine 0·64 mg.
1	(3) 1076	(2) 1030	(5) 11	(6) 3
		Strain B		Strain B
			Mepacrine 0·64 mg.	Mepacrine 0·64 mg.
2	(3) 948	(3) 574	(5) 143	(3) 118
			Pamaquin 0·08 mg.	Pamaquin 0·08 mg.
			(6) 10	(3) 0
		Strain B		Strain B
			Mepacrine 0·64 mg.	Mepacrine 0·64 mg.
3	(5) 1002	(6) 699	(8) 7	(5) 44
			Pamaquin 0·08 mg.	Pamaquin 0·08 mg.
			(6) 0	(8) 11
		Strain A		Strain A
			M. 4430 0·4 mg.	M. 4430 0·4 mg.
4	(2) 805	(2) 841	(4) 0	(4) 640
		Strain B		Strain B
				M. 4430 0·4 mg.
		(3) 494		(6) 834

The intensity of infection is expressed as the average number of parasites per 500 erythrocytes in a group of birds. The number of birds in each group is given in parentheses.

No significant difference in the intensity of infection as compared with the normal strain was observed in a group of birds infected with Strain A (resistant to 0·4 mg./20 g. of paludrine) and treated with 0·64 mg. of mepacrine (Table 5, exp. 1) (Bishop & Birkett, 1947). As Strain A was resistant only to 0·4 mg. of paludrine it seemed possible that Strain B, resistant to a higher dose of the drug (1·0 mg./20 g.), might possess a resistance to mepacrine which had not been induced by treatment with the lower doses of paludrine. The reaction of Strain B to mepacrine was therefore compared with that of the normal strain (Table 5, exps. 2 and 3). In both experiments considerable variation in response to

of *P. gallinaceum* also was compared (Table 5, exps. 2 and 3), but no significant difference in sensitivity to the drug was observed. Paludrine-resistance therefore confers no resistance to pamaquin.

It seemed possible that though enhanced resistance to paludrine does not include an increase in resistance to compounds which differ from it as fundamentally in chemical constitution as mepacrine and pamaquin, it might extend to compounds in which this difference is only slight. M. 4430 (N^1-*p*-chlorophenyl-N^5-methyl-N^5-isopropylbiguanide), the methyl homologue of paludrine (Curd, Davey & Rose, 1945*c*), has a pronounced antimalarial effect upon *P. gallinaceum*, its minimum effective dose

being 0·4 mg./20 g., as compared with 0·1 mg./20 g. of paludrine (Davey, 1946*a*). A course of 0·4 mg./20 g. of M. 4430 twice daily for 3½ days was found to inhibit the development of the normal strain of *P. gallinaceum* in chicks (Table 5, exp. 4), whereas it was without effect upon the paludrine-resistant strains A and B. It is to be concluded therefore, as stated in our preliminary account (Bishop & Birkett, 1947), that resistance to paludrine confers resistance to the methyl homologue of this compound, but whether it extends to other biguanides has not been ascertained. Williamson, Bertram & Lourie (1947) have also found, independently, that a strain of *P. gallinaceum* resistant to paludrine was also resistant to 4430, though not to 3349 or 3502, both of which are earlier derivatives of the series of compounds which led to the synthesis of paludrine and which differ from that compound and from 4430 in the presence of a pyrimidine ring (Curd, Davey & Rose, 1945*c*).

VIII. THE IMMUNOLOGICAL RELATIONSHIP OF THE PALUDRINE-RESISTANT AND NORMAL STRAINS OF *PLASMODIUM GALLINACEUM*

It has long been known that, in avian malaria, the reinoculation with blood heavily infected with parasites of the same species fails to produce an acute attack in birds which are carrying a latent infection (Wasielewski, 1902; Moldovan, 1912). Later, it was shown (Taliaferro & Taliaferro, 1929) that when washed parasitized cells of *P. cathemerium* are introduced intravenously into a canary with a latent infection of the same species they are removed from the blood stream within a short period of time. Similar immunity to superinfection has been shown to exist in *P. gallinaceum*.

In order to discover whether the development of resistance to a drug affects the immunological reaction of *P. gallinaceum* the following series of experiments was performed.

A bird was inoculated intravenously with blood heavily infected with the normal strain of *P. gallinaceum*. When the acute infection had passed into a latent stage and few or no parasites were seen in the peripheral blood, the bird was inoculated intravenously with blood heavily parasitized with the paludrine-resistant strain (Strain B). After 15 min. the recipient showed a parasite count of 90 per 500 corpuscles. Within 48 hr. the parasites had been removed from the blood stream, and none was found on a 3 min. examination of the blood.

A second bird, inoculated intramuscularly with sporozoites of the normal strain of *P. gallinaceum*, developed a heavy infection which passed into a latent condition, few parasites being found on a 3 min. examination of the blood. The bird was inoculated intravenously with blood heavily infected with the paludrine-resistant strain (Strain B). 15 min. after the inoculation the number of parasites per 500 corpuscles was found to be 120; but within 24 hr. it had dropped to 1 per 500.

A converse experiment was then performed, in which a chick was inoculated with sporozoites of the paludrine-resistant strain (Strain B). When the infection had passed into a chronic state in which few or no parasites were found on a 3 min. examination of the blood, the bird was inoculated intravenously with blood heavily infected with the normal strain of *P. gallinaceum*. A parasite count made 15 min. later showed 61 parasites per 500 corpuscles. Within 24 hr. the parasites had been removed from the blood stream, and the parasite count fell to 0 per 500 corpuscles.

These experiments therefore prove that the development of resistance to paludrine does not affect the immunological reaction of *P. gallinaceum*: a latent infection of the drug-resistant strain protecting against the normal strain, and the normal strain protecting against the drug-resistant strain.

IX. THE EFFECT OF MOSQUITO-TRANSMISSION UPON PALUDRINE-RESISTANCE IN *PLASMODIUM GALLINACEUM*

Though the development of enhanced resistance to drugs in blood-inoculated infections of malaria is a matter of some biological interest, the question whether this resistance persists after cyclical development in the mosquito raises wider and more fundamental issues. A series of experiments, therefore, was performed to determine whether the paludrine-resistant strain of *P. gallinaceum* is infective to *Aëdes aegypti*, and if so, whether infections induced in chicks by sporozoites of this strain are paludrine-resistant.

It had been observed that in the normal strain of *Plasmodium gallinaceum* passaged for a long period by intravenous injection at short intervals of time, the proportion of gametocytes to asexual parasites was very low, although a good deal of variability was seen between individual birds. With such infections in which the gametocyte population is sparse the rate and intensity of infection produced in *Aëdes* is poor. In the majority of chicks infected with the paludrine-resistant strain the proportion of gametocytes to asexual parasites was greater than in the normal strain, though both strains were maintained by the same method of passage. It was noticed that in the paludrine-resistant strain, when the dose of drug was increased and the strain was, for the time being, adversely affected by the drug, the gametocytes were numerous and normal in appearance, whereas the asexual parasites, particularly the

mature schizonts, were few and frequently were abnormal in appearance. In this lack of response of the gametocytes to paludrine *Plasmodium gallinaceum* resembles *P. vivax* and *P. falciparum*. Maegraith, Adams, King, Townshend, Davey & Harvard (1945) had noted in cases of malignant tertian malaria that the gametocytes were unaffected by doses of paludrine which cleared the blood of asexual forms, and these observations were confirmed in this species and in *P. vivax* by Fairley (1946).

Fairley and his co-workers (1946) have shown that though the gametocytes were not affected by paludrine, the presence of small quantities of the drug in the infected blood inhibits the development of *P. vivax* and *P. falciparum* in the mosquito. Though exflagellation and fertilization may take place, the development of the oocysts on the gut-wall is arrested at an early stage. It seemed possible, therefore, that although normal gametocytes were present in paludrine-resistant strains of *P. gallinaceum*, their development in the mosquito might be inhibited if the drug were present in the blood of the host. That this is not the case was shown by the presence of heavy infections of sporozoites in the salivary glands of mosquitoes fed upon chicks infected with the paludrine-resistant strain and receiving twice-daily doses of 0·4 or 1·0 mg./20 g. of paludrine. The rate and intensity of infection, in mosquitoes gorged upon birds which had received paludrine in these doses 3, 4 or 5 hr. prior to being bitten, was high. The presence of paludrine in the blood of the chick, in doses many times greater than the minimum dose effective upon normal erythrocytic infections of *P. gallinaceum*, has no inhibitive effect, therefore, upon the developmental cycle of the paludrine-resistant strain in the mosquito.

In the first experiment designed to determine whether paludrine-resistance persists after cyclical development in the mosquito, a strain of *P. gallinaceum* resistant to 0·2 mg./20 g. of paludrine was used. A batch of *Aëdes aegypti* was fed upon an infected chick which had received a 3½-day course of treatment with 0·1 mg. of paludrine twice daily, the last dose being given 48 hr. before the mosquitoes were allowed to feed. The glands of six out of eight of the mosquitoes were heavily infected with sporozoites when examined on the 10th day. When these sporozoites were injected into a chick intravenously and the bird treated twice daily with 0·1 mg. of the drug, uninucleate parasites appeared in the peripheral blood on the 6th day. By the 7th day a heavy infection of parasites in all stages of development was present, although drug treatment was still being given.

This experiment was repeated with a strain of *Plasmodium gallinaceum* resistant to 0·4 mg. of paludrine (Strain A). The infected chick upon which the mosquitoes were fed had received a course of four twice-daily doses of drug, the last dose being given 4 hr. before the mosquitoes were allowed to gorge. The salivary glands of twelve out of thirteen of the mosquitoes were found to be infected, the majority heavily, when examined on the 13th day, although the number of gametocytes present in the chick had not been great. A suspension of sporozoites was injected intravenously into a chick treated from the day of inoculation with twice-daily doses of 0·4 mg. of paludrine, and an infection normal in intensity and appearance developed on the 6th day.

It had been observed (Bishop, Birkett & Gilchrist, 1947) in sporozoite-induced infections of *P. relictum* in canaries, that erythrocytic parasites, arising from exo-erythrocytic forms, develop into mature schizonts and gametocytes during treatment with doses of quinine or mepacrine suppressive to blood-inoculated infections. The development of an erythrocytic infection, during treatment with paludrine, in sporozoite-induced infections of *P. gallinaceum* could not therefore, without further test, be accepted as proof of drug-resistance. In the following experiments the resistance to paludrine was studied in blood-inoculated infections of paludrine-resistant strains which had undergone cyclical development in mosquitoes. A batch of mosquitoes was fed upon a bird infected with Strain A of *P. gallinaceum* resistant to 0·4 mg./20 g., or Strain B resistant to 1·0 mg./20 g. of paludrine. A suspension of the sporozoites which developed was injected intravenously into an untreated bird. When the infection became patent the bird was bled, and a series of birds inoculated intravenously, each with 50,000,000 parasites. The intensity of infection produced by the paludrine-resistant strain after one mosquito passage was compared with that of the normal strain both in birds treated with paludrine and in untreated birds (Table 6). In the first three experiments the sensitivity of Strain A to 0·4 mg./20 g. of paludrine was tested after one mosquito passage. Parasites normal in appearance developed in all the birds inoculated with the paludrine-resistant strain, whereas infections of the untreated strain were suppressed by the drug. Although the infections produced in Exp. 3 were light, the parasites were normal. In Exp. 4 the sensitivity of Strain A was tested not only to 0·4 mg., but to 0·2 mg. and 0·1 mg./20 g. of paludrine. The infections which developed in birds treated with 0·1 mg. were heavy. Williamson, Bertram & Lourie (1947) have also found that paludrine-resistance persisted after a single mosquito passage.

As resistance to paludrine was retained after a single transmission through mosquitoes, the effect of further mosquito transmissions without intervening drug treatment was studied. Strain A, resistant to

0·4 mg. of paludrine, was passed three times through chicks and mosquitoes alternately (Table 6). The period of time covered by the passages during which no drug treatment was given was 59 days. The sensitivity of the strain to 0·4 and 0·2 mg. of paludrine was tested by the usual method after the 3rd mosquito passage, and resistance to the drug was shown still to be present.

of paludrine (Table 6). The strain was proved to have retained its resistance to the drug.

These experiments therefore show that resistance to paludrine is, within the limits tested, of a stable nature, and that it is not lost during five serial transmissions through the mosquito vector. This involves not only a change of habitat, but a period of 3 months without contact with the drug.

Table 6. *Comparison of the reaction of paludrine-resistant and normal strains of* Plasmodium gallinaceum *to paludrine HCl*

		Untreated birds		Treated birds	
No. of exp.	Dose twice daily	Normal strain	Paludrine-resistant strain	Normal strain	Paludrine-resistant strain
		Strain A after one mosquito passage			
1	0·4	(3) 1076	(2) 979	(5) 1	(5) 540
2	0·4	(6) 1001	(6) 903	(6) 0	(6) 466
3	0·4	(3) 1075	(3) 617	(6) 1	(5) 158
4	0·4	(4) 559	(5) 544	(4) 0	(5) 398
	0·2	—	—	—	(6) 537
	0·1	—	—	—	(4) 720
		Strain A after three mosquito passages			
5	0·4	(6) 1240	(5) 685	(6) 0	(6) 268
	0·2	—	—	—	(6) 451
		Strain A after five mosquito passages			
6	0·4	(6) 1344	(6) 862	(6) 1	(6) 398
	0·2	—	—	—	(6) 784
	0·1	—	—	—	(6) 945
		Strain B after one mosquito passage			
7	1·0	(3) 948	(5) 1081	(6) 1	(5) 246
	0·8	—	—	—	(5) 624

The intensity of infection is expressed as the average number of parasites per 500 erythrocytes in a group of birds. The number of birds in each group is given in parentheses.

The effect of five passages of the A strain through mosquitoes, with intervening passages through chicks, was also studied. The total period of time covered by these mosquito and chick passages, during which no drug treatment was given, was 3 months, but resistance to paludrine was proved to have persisted (Table 6) (Bishop & Birkett, 1947).

A batch of *Aëdes aegypti* was fed upon a chick infected with Strain B of *Plasmodium gallinaceum*, resistant to 1·0 mg./20 g. of paludrine, and although the bird was undergoing treatment with that dose of drug, the last dose having preceded the gorging of the mosquitoes by 3–4 hr., the glands of all the eight mosquitoes were heavily infected with sporozoites when examined 10 days later. The resistance of the parasites after this mosquito passage was tested with 1·0 and 0·8 mg./20 g. doses

X. DISCUSSION

As a result of continuous treatment with gradually increasing doses of the drug, a forty-fold increase in resistance to paludrine has been developed in 4½ months, by *Plasmodium gallinaceum* in chicks (Bishop & Birkett, 1947). Attempts to increase this resistance further were limited by the toxicity of the drug to the host. In the rapidity with which resistance developed, and its intensity, our results are essentially similar to those obtained independently by Williamson *et al.* (1947). The comparative ease with which resistance to paludrine was induced contrasts markedly with the complete failure to induce resistance to mepacrine, even after prolonged treatment with the drug; and the slowness of the development of a low grade resistance to pamaquin. Whereas Williamson, Bertram and Lourie failed to

induce resistance in *P. gallinaceum* to sulphadiazine, we have succeeded in producing a strain resistant to this drug by the method used in producing resistance to paludrine and pamaquin. The speed with which resistance to sulphadiazine has developed has been slower than that at which resistance to paludrine developed, but not so slow as the development of resistance to pamaquin, and it is gradually increasing in intensity. A full account of these results will be published later, when resistance has reached its maximum.

Variability in the speed with which drug-resistance is developed was observed by Yorke, Murgatroyd & Hawking (1932) in trypanosomes. Thus the time taken to produce maximum resistance in *Trypanosoma rhodesiense* varied from 4 to 8 weeks with atoxyl, arsacetin, tryparsamide, reduced tryparsamide thioglycollate, stibenyl, and acriflavine, to 12 months or more with Bayer 205; though there was an indication that the rapidity of development of resistance varied with the particular technique used. With the diguanide compound, synthalin, and with undecane diamidine the development of resistance was slow as compared with aromatic arsenicals, antimonials and acriflavine (Lourie & Yorke, 1938).

Whereas paludrine, sulphadiazine, pamaquin and mepacrine are all highly effective upon erythrocytic parasites of *Plasmodium gallinaceum*, mepacrine is without demonstrable effect upon the exo-erythrocytic cycle of development. It is interesting to note that the three compounds with which we have succeeded in producing drug-resistance are all known to have some action upon exo-erythrocytic forms of this species. Thus Coggeshall, Porter & Laird (1944) found that sulphadiazine had a marked effect upon the exo-erythrocytic parasites of *P. gallinaceum*, though Davey (1946*b*) found that its action was slower than that of paludrine which had a very pronounced effect. Coggeshall and his co-workers and Davey have all found that the action of pamaquin upon exo-erythrocytic forms is very slight. The facility with which these compounds produce resistant strains of *P. gallinaceum* appears to be comparable with the intensity of their activity upon exo-erythrocytic forms. It is, however, improbable that the development of resistance to a drug is due to its action upon this particular phase in the developmental cycle, as the method used to induce it has been one which limits the development of exo-erythrocytic forms to a minimum. Also, whereas we have, by this method, obtained a slight but definite degree of resistance to pamaquin, Fulton (1942) failed to induce resistance to this compound under conditions which would allow an unrestricted development of the exo-erythrocytic forms, and Williamson *et al.* (1947) have failed to induce resistance to sulphadiazine.

Although it is improbable that the development of a drug-resistant strain of *P. gallinaceum* is due to the action of the drug upon exo-erythrocytic forms, it has been shown (p. 130) that the exo-erythrocytic parasites of a paludrine-resistant strain are themselves resistant to the drug. It has also been shown that paludrine-resistance persists after passage through the mosquito. This involves development in an environment entirely different from the erythrocytes. The resistance, once developed, is therefore of a very stable nature, and is not lost when a fundamental change in the environment occurs which, presumably, entails a change in the method of nutrition of the parasite.

It is obvious from our results, and from those of Williamson, Bertram and Lourie, that a strain of *P. gallinaceum* resistant to paludrine is not thereby made resistant to all anti-malarial compounds. We have ourselves found that the paludrine-resistant strain is sensitive to mepacrine and pamaquin, and Williamson and his co-workers have shown that their paludrine-resistant strain retained its normal sensitivity to quinine, mepacrine, sulphadiazine, and the pyrimidine compounds 3349 and 3502. Resistance to paludrine is not, however, specific to that compound, but extends to the methyl homologue M. 4430. No data have, as yet, been obtained to show the extent of the changes which can be made in chemical constitution without loss in resistance. That in trypanosomes resistance may extend to members of a group of related compounds has been shown by Yorke, Murgatroyd & Hawking (1932), who found that in *Trypanosoma rhodesiense* resistance to an aromatic arsenical or antimonial compound implied resistance to other aromatic arsenicals and antimonials, though not to non-aromatic compounds of arsenic and antimony, nor to Bayer 205. In bacteria resistance to one sulphonamide has been found to confer resistance to other members of the group. Thus Sesler & Schmidt (1942) found that pneumococci which had developed resistance to either sulphanilamide, sulphapyridine or sulphathiazole were resistant to each of the remaining drugs and to sulphadiazine; and Lankford, Scott & Cooke (1943), and Kirby & Rantz (1943) have obtained similar results. Such information is important not only from a clinical point of view, but for the light it may throw upon the mode of action of the drugs. It is interesting in this connexion to note that whereas in *Plasmodium gallinaceum* some degree of resistance to pamaquin has been obtained, all attempts to produce resistance to mepacrine have failed, though the same experimental methods have been used. These two compounds have the same N-diethylamino-isopentyl-8-amino side chain, though mepacrine is an acridine derivative and pamaquin is a quinoline compound.

Whereas any light which experiments upon drug-resistance may throw upon the mode of action of anti-malarial compounds, and upon the relationship of chemical constitution and anti-malarial activity, is important, the observations which are of the greatest importance biologically are those which prove that paludrine-resistance is retained after mosquito-transmission. In naturally acquired malaria it is the mosquito which transmits the infection from one vertebrate host to another, and, under such conditions, the dispersal of a drug-resistant strain of parasite would depend upon whether the resistance persists after passage through the mosquito. It must be stressed, however, that our experiments proving that paludrine-resistance is retained after mosquito transmission are based upon laboratory experiments with avian malaria, and may be devoid of any significance in human malaria. Apart from any question of dispersal, the mosquito-transmission of a drug-resistant strain is of fundamental importance biologically, as it raises an issue which, with the possible exception of the observations (p. 125) of Kritschewski & Halperin (1933), no other work upon drug-resistance in parasitic organisms has raised. Hitherto the extensive literature upon drug-resistance has dealt with organisms in which no sexual cycle is known, e.g. bacteria and trypanosomes. The work of Yorke, Murgatroyd & Hawking (1933*a*, *b*), proving that strains of *Trypanosoma brucei* resistant to aromatic arsenicals were infective to *Glossina*, and that the resistance was retained after two successive passages through the flies, is of importance in the treatment of trypanosomiasis. The developmental cycle of trypanosomes does not, however, include a sexual phase in either the vertebrate or invertebrate host. In this it differs from that of malaria in which fertilization occurs in the gut of the mosquito. The work upon *Plasmodium gallinaceum* has now shown that in this organism at least, drug-resistance is not a character which is merely transmitted during asexual reproduction, but is retained after sexual reproduction of the parasite.

XI. SUMMARY

1. Attempts, made over a period of more than 6 months, to produce a strain of *Plasmodium gallinaceum* resistant to mepacrine failed.

2. A strain of *P. gallinaceum* resistant to daily doses of 0·08 mg./20 g. of pamaquin was obtained after approximately 8 months' treatment with the drug.

3. A strain of *P. gallinaceum* resistant to the maximum dose of paludrine which the chicks would tolerate (1·0 mg./20 g. twice daily) was obtained after 4½ months' treatment with gradually increasing doses of the drug.

4. The exo-erythrocytic parasites of *P. gallinaceum* formed in chicks infected with a paludrine-resistant strain are themselves resistant to the drug. It is not, however, considered probable that the development of resistance is due to the action of the drugs upon the exo-erythrocytic parasites, as the technique used was one which reduced their number to a minimum.

5. A strain of *P. gallinaceum* resistant to paludrine showed a normal sensitivity to mepacrine and pamaquin, but was resistant to 4430, the methyl homologue of paludrine.

6. Chicks carrying a latent infection of the normal strain of *P. gallinaceum* were immune to infection with the paludrine-resistant strain; and conversely chicks carrying a latent infection of the paludrine-resistant strain were immune to infection with the normal parasite.

7. A paludrine-resistant strain of *P. gallinaceum* retained its resistance to this drug after five cyclical passages through mosquitoes without intervening drug treatment.

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ON LARVAE AND NYMPHS OF SOME SPECIES OF PALESTINIAN *HYALOMMA**

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(With 12 Figures in the Text)

INTRODUCTION

The available knowledge of adults in the genus *Hyalomma* is confused and contradictory, and it is therefore not surprising that there is hardly any reliable information on the pre-imaginal stages. The only species which has been thoroughly investigated is *H. dromedarii* studied by Delpy (1937). A key to the larvae and nymphs found on cattle in Algeria has been prepared by Senevet (1925, 1928) and includes the species *H. mauritanicum*, *H. lusitanicum* and *H. aegyptium*, but this author did not give any exact description. Chodziesner (1924) has given an incomplete description of *H. aegyptium*, *H. syriacum* and *H. scupense*. The descriptions of the same species given by different authors do not agree and it is impossible to compare them; it is even likely, in view of the difficulty in determining adults, that they were working with different species.

In the present paper a description of the pre-maginal stages of *H. savignyi*, *H. dromedarii*, *H. marginatum* and *H. detritum* raised from females, determined by the method described in a previous publication (Adler & Feldman-Muehsam, 1948), is given as well as some information on their biology.

Hyalomma savignyi

Unfed larva. The general form of the larva is elliptic, length 0·54 mm. and width 0·42 mm.

The colour is brownish yellow. The scutum is brown; length 240 μ. There is a ring of dark-brown-red pigment round the eyes (Fig. 1*a*). Under the high power the surface of the scutum appears granular. The basis capituli has the form of a triangle, with its base posterior (Fig. 2*a*). The two angles at the posterior end have a systematic value. In other species they are more obtuse (Fig. 2 *b*, *c*).

The palps have three segments; the second segment corresponds to two and three of adult and nymph. The width of the hypostome is 36·4 $\pm$ 5 μ (twenty-eight specimens measured).

There is a short median triangular spur on the medial part of the first coxa (Fig. 3*a*). This is not to be confused with the larger internal spur which serves for muscular attachments and is seen only in cleared specimens; it lies almost directly over the external spur.

The information in the literature on the stigmata and tracheal system of the larva is very contradictory. Vogel (1927) says that there are no stigmata in the larva. Delpy (1937) describes one pair of small stigmata behind the coxa of the third pair of legs. Other authors do not mention the stigmata at all. As a matter of fact, four pairs of a structure corresponding to stigmata are found in the larva: the first three behind the coxa of every pair of legs, and the fourth on the dorsal side of the alloscutum, almost on the posterior end of the larva (Fig. 4). Since no tracheal system has as yet been detected in the larva, it would appear that these stigmata are not functional.

Nothing is mentioned in the literature on the dorsal porosa of the larva; but this sense organ is found not only in the adult tick, as previously believed, but also in the nymph and the larva. In the nymph the dorsal porosa consists of two to five sensory elements: in the larva there is only one (Fig. 5). Each element of the dorsal porosa is surrounded by a fine granular ring in the adult and also in the pre-imaginal stages. It is this ring which distinguishes the area porosa in the larva from other minute sense organs on the surface (Fig. 5).

The form and length of the scutum varies according to the species (Fig. 1). The length of the scutum of *H. savignyi*, measured from the scapula to its posterior end, is 240 $\pm$ 1 μ (twenty-eight specimens examined). The structure of the scutum is clearly seen in mounted specimens of exuviae of the larvae (also of the nymphs). These mounted specimens show all the details of the structure without any artifacts, as it is not necessary to put the exuviae through KOH. (They may be put directly into 70° C.

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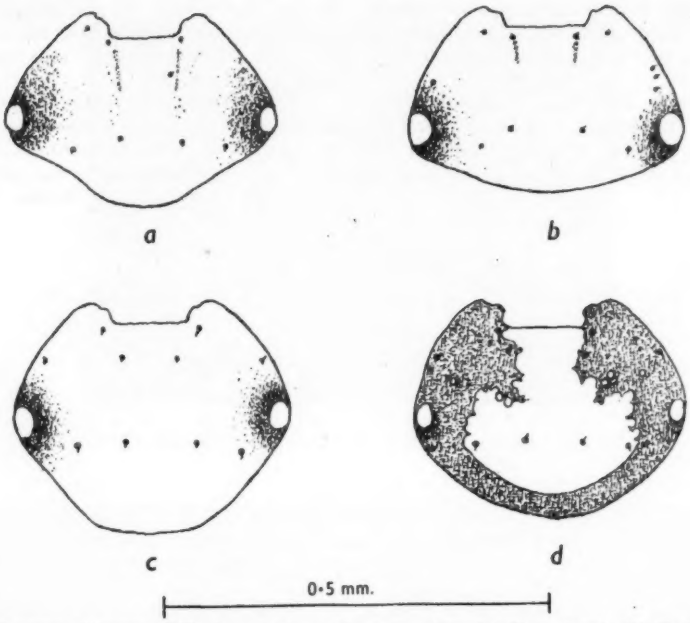

Fig. 1. Scutum of larvae of *Hyalomma*, drawn from exuviae. (*a*) *H. savignyi*; (*b*) *H. dromedarii*; (*c*) *H. marginatum*; (*d*) *H. detritum*.

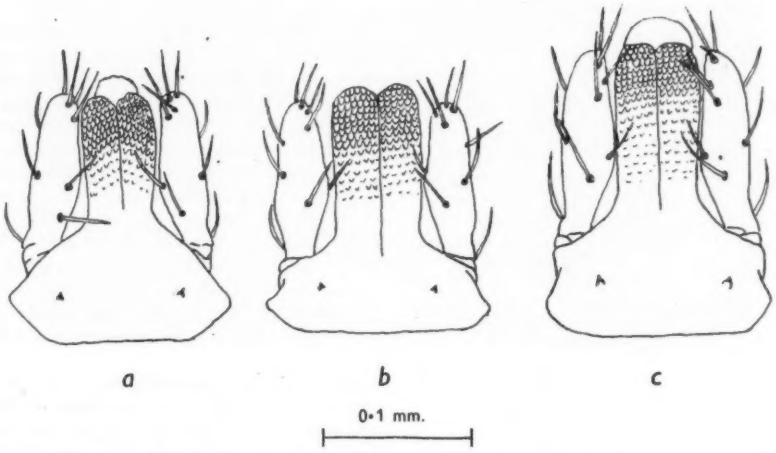

Fig. 2. Capitulum of larvae of *Hyalomma*. (Dorsal aspect.) (*a*) *H. savignyi*; (*b*) *H. marginatum*; (*c*) *H. detritum*.

alcohol. It is advisable to heat the alcohol slightly in order to expel the air from the legs.)

The gorged larva. The gorged larva measures c. 1·7 × 1·1 mm. Its colour is red, white or yellowish according to whether it has fed on blood or lymph.

The nymph. The colour of the nymph is yellowish brown. The scutum is darker than the rest of the larva. The basis capituli has a similar form to that of the larva. The palps are 4-jointed, slender and long. The hypostome is $78 \pm 1\,\mu$ in width (twenty-eight specimens measured). The scutum is somewhat wider than long: 0·56 × 0·58 mm. The first coxa has

two long spurs (Fig. 7*a*). The areae porosae dorsalis are very small. Each plate contains two to four sensory elements, and is surrounded by a circle of fine points (Fig. 6).

The gorged nymph. The gorged nymph measures *c.* 5·4 × 3·8 mm. Its colour is bluish grey, white, yellowish white or pale rose, depending on the relative quantities of blood and lymph imbibed (as with the larvae).

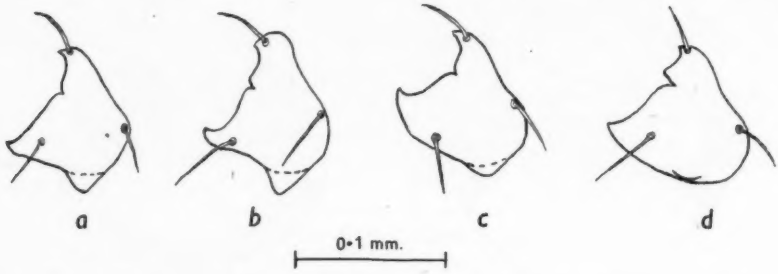

Fig. 3. First coxa of larvae of *Hyalomma*. (*a*) *H. savignyi*; (*b*) *H. dromedarii*; (*c*) *H. marginatum*; (*d*) *H. detritum*.

The exuvia of the nymph. The exuvia is very helpful in the study of the scutum. The chitin of the scutum is thick and reddish brown in colour. The central area between the cervical grooves is much clearer and yellowish. The alloscutum of the exuvia is thin and white. In the exuvia the chitinous extensions of the scutum to which the muscles were attached become obvious. It is characteristic for each species (Fig. 12).

Biology of H. savignyi. This is the most common member of the genus in Palestine. It was found throughout the whole country. It appears in large numbers in the beginning of the summer and is the last to disappear. (In the Jordan Valley and the Negeb the ticks are found on cattle during the whole winter.) It is also found in large numbers on camels, and rarely on goats. We have found gorged specimens of all stages from larvae to adults on cows and camels.

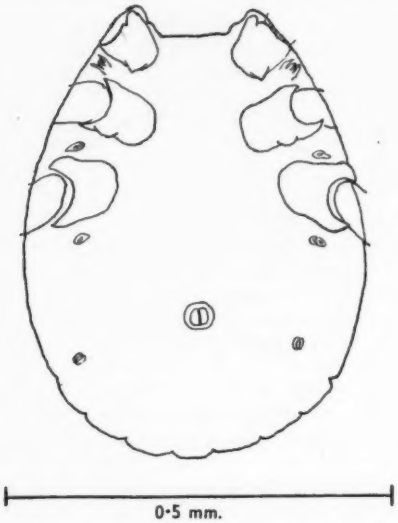

Fig. 4. Ventral side of larva of *H. savignyi* showing the four pairs of stigmata. The fourth pair is dorsal and can be seen from the ventral aspect in cleared specimens.

It is easy to raise *H. savignyi* in the laboratory on hedgehogs. Breeding was carried out in the following way. Hedgehogs were kept in cages with a floor of wire network. The cages were put in a tray which was placed in another larger tray containing water. This arrangement prevented the escape of the ticks. The larvae and nymphs as they leave the hedgehog may be picked up in the inner tray or in the water, from which they can be collected unharmed, and used for experiments or breeding as required. The larvae of *H. savignyi* remain on the hedgehog for an average of 4–6 days. The fed larvae were kept in test-tubes till ecdysis, and some days later they were replaced as nymphs on a hedgehog for feeding.

H. savignyi is generally a 3-host tick, but occasionally the gorged larvae remain on the host and descend only as gorged nymphs. The larvae were kept at 32° C. and about 75 % R.H. They were not put on the hedgehog for feeding until they were 2–4 weeks old (younger larvae do not feed). Ecdysis occurs about 6 days after the larva leaves the host at a temperature of 30° C., 4–5 days at 38° C. and 30–50 days at 17·5° C. Nymphs do not feed till they are about 1 week old, and an average feed lasts for 9–12 days. The time which elapses from the descent of the gorged nymphs until the emergence

Fig. 5. Areae porosae dorsalis of larva of *H. savignyi*. × 1060.

Fig. 6. Areae porosae dorsalis of nymph of *H. savignyi*. × 600.

of the imagines varies within very wide limits; from 11 to 24 days for females, and 12 to 35 days for males at 35° C. At 30° C. it varies between 11 and 56 days for males and females.

It seems that in nature there are at least two generations of *H. savignyi* in a year. The duration of one generation at 32° C. was estimated to be 116 days. This estimate was based on the following figures, which are averages obtained in the laboratory:

	Days
Egg stage	21
Larva (from emergence to feeding)	14
Duration of feed	5
From leaving host to ecdysis	6
Nymph (from emergence to feeding)	7
Duration of feed	10
From leaving host till ecdysis	25
Imago (from emergence to feeding)	14 (?)
Duration of feed	7 (?)
From completion of feed till oviposition	7

Under field conditions during summer some of these stages may be shorter, but the opportunities for finding a suitable host for the larvae or nymphs may depend on accidental factors. At a temperature of 30° C. and R.H. 80 % the larvae can survive for 56 days without feeding. Galuzo (1939–41), working in the Kazakh under field conditions, also concluded that there are several generations in a year.

from those of the other species because it is longer and relatively narrower (Fig. 9*a*). The scutum of the gorged larva is darker than that of the ungorged, and has a red-brown colour, especially near the margins; there is a dark outer zone producing a characteristic appearance (Fig. 1*d*).

The unfed nymph. The unfed nymph is easily distinguished even with the naked eye from the other

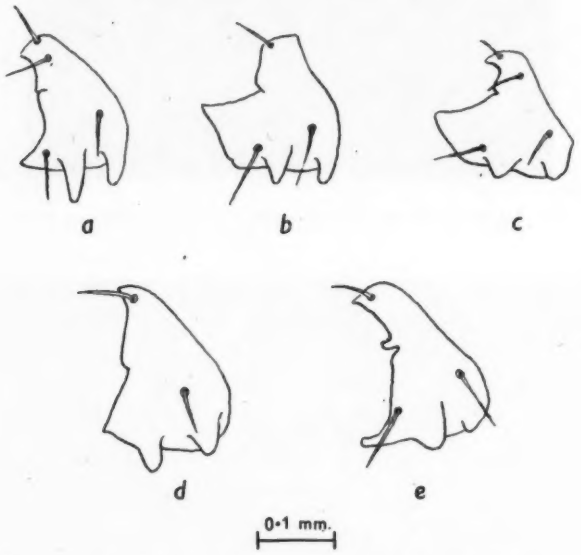

Fig. 7. First coxa of nymphs of *Hyalomma*. (*a*) *H. savignyi*; (*b*) *H. dromedarii*; (*c*) *H. aegyptium*; (*d*) *H. marginatum*; (*e*) *H. detritum*.

Hyalomma detritum

The unfed larva. The unfed larva is similar in its general aspect to that of *H. savignyi*. The size is about 0·55 mm. (without capitulum) × 0·45 mm. The scutum is rounded (Fig. 1*d*), brown in colour, and has a granular structure similar to that of *H. savignyi*; length 280 ± 1 μ (twenty-eight specimens measured). The postero-external extremities of the basis capituli are more obtuse than in *H. savignyi* (Fig. 2*c*). The capitulum as a whole is longer and stouter. The palps are long and wide. The hypostome is relatively very large 46 ± 0·06 μ (twenty-eight specimens measured, Fig. 8*d*). The first coxa has a very small rounded almost unnoticeable spur (Fig. 3*d*). The stigmata are as in *H. savignyi*, but the fourth pair is not on the dorsum but on the margin of the alloscutum. Areae porosae dorsalis as in *H. savignyi*.

The gorged larva. It is very easy to distinguish with the naked eye gorged larvae of *H. detritum*

nymphs studied. It is relatively narrow and long and the capitulum and scutum have a transparent white yellowish colour. The legs are almost transparent. The alloscutum has a characteristic red-brown colour, especially in its posterior end. The areae porosae dorsalis are relatively large and very conspicuous. Each area contains three to five sensory elements. The scutum has a granular structure similar to that of the larva, and is rounded (Fig. 12*c*); length 600 ± 7 μ (twenty-eight specimens measured). The first coxa bears one long spur and another very small one (Fig. 7*e*). The palps are long and wide (Fig. 11*b*). The hypostome is relatively wide (at the broadest part 88 ± 1 μ in twenty-eight specimens measured) (Fig. 10*b*).

The gorged nymph. The gorged nymph is also longer and narrower than that of *H. savignyi*. It measures c. 5·7 × 3·4 mm. (without capitulum). The colour is greenish brown.

The exuvia of the nymph. The exuvia is characteristic. The alloscutum is thick and not trans-

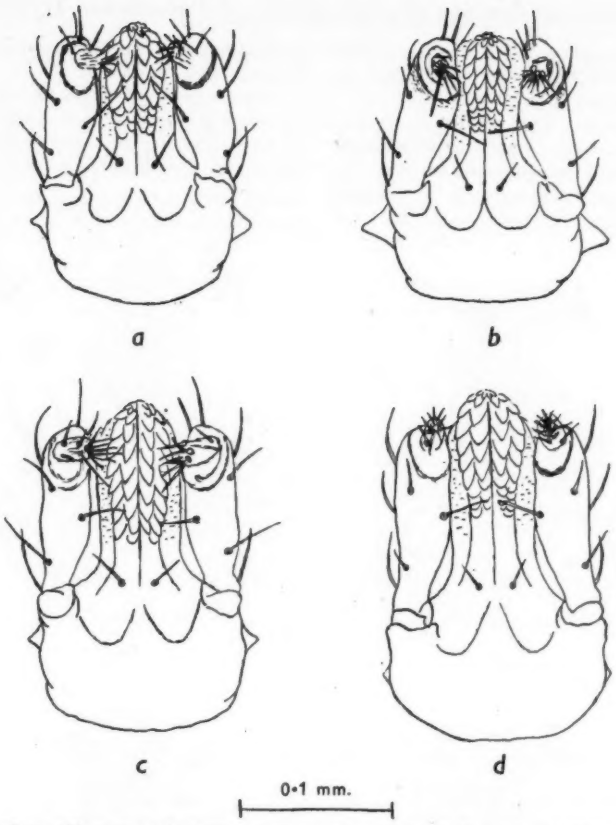

Fig. 8. Capitulum of larvae of *Hyalomma*. (Ventral aspect.) (*a*) *H. savignyi*; (*b*) *H. dromedarii*; (*c*) *H. marginatum*; (*d*) *H. detritum*.

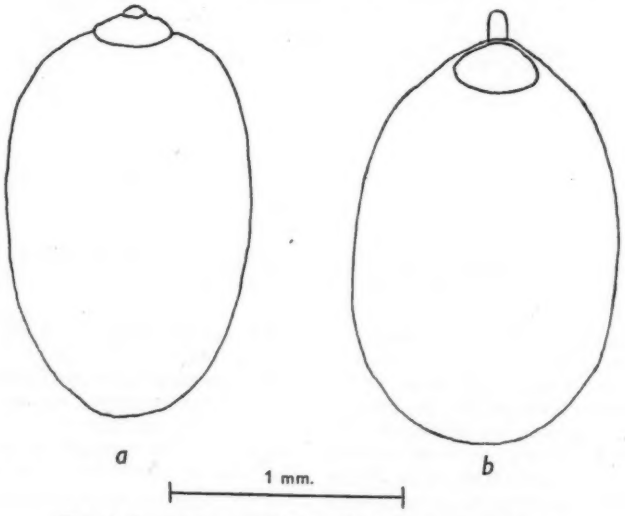

Fig. 9. Gorged larvae of (*a*) *H. detritum*; (*b*) *H. savignyi*.

parent, and has a dark-yellow colour. The form of the scutum is clearly seen (Fig. 12*c*). The lateral extension of the scutum is typical (Fig. 12*c*).

Biology of H. detritum. This species is not very common in Palestine. It was found on cattle for the first time in the valley of Esdraelon (where the genus *Hyalomma* is extremely widespread). The adults and rabbits and breeding experiments indicate that it is a 2- and 3-host tick.

From a group of unfed larvae that were put on a hedgehog twenty-five descended as gorged larvae; sixty as unfed nymphs; and a few as gorged nymphs. The larvae feed usually for 7–8 days, occasionally up to 14 days.

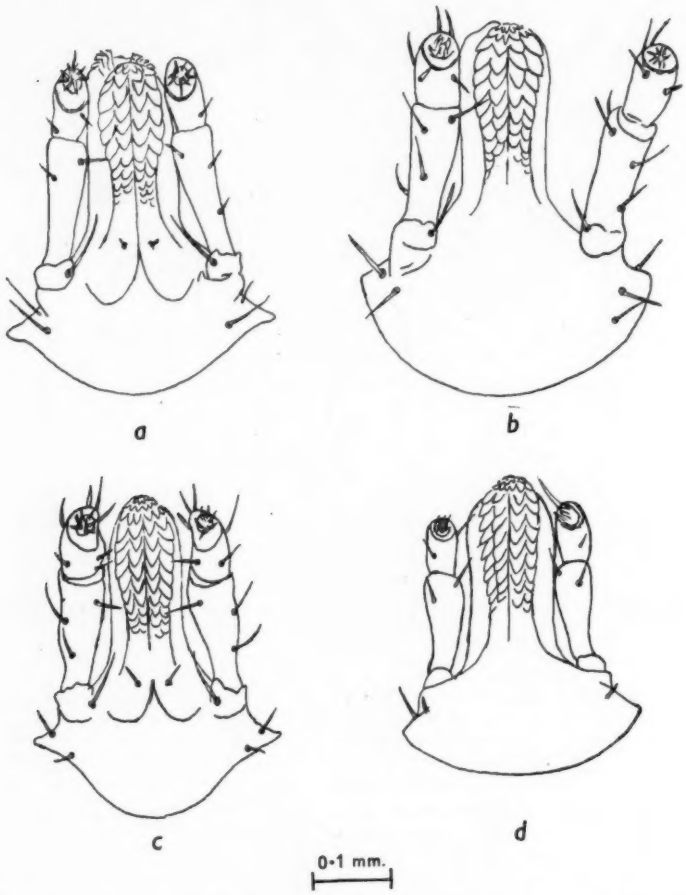

Fig. 10. Capitulum of nymphs of *Hyalomma*. (Ventral aspect.) (*a*) *H. savignyi*; (*b*) *H. detritum*; (*c*) *H. aegyptium*; (*d*) *H. marginatum*.

seem to appear only in the middle of the summer and not in great numbers. *H. detritum* was also found on cattle in the slaughter-house in Jerusalem in the beginning of the summer (the origin of the cattle is unknown). Apart from cattle a few individuals were also found on camels. We have as yet no information on the bionomics of this species in the field. In the laboratory *H. detritum* can be reared on hedgehogs

The nymphs feed for 6–9 days. The period from leaving the host to ecdysis (Delpy's 'nymphal sleep') varies within wide limits; for females from 16 to 33 days at 35° C. and from 17 to 80 days at 32° C. (data obtained from the offspring of one female). On the average the 'nymphal sleep' lasts for about 27 days at 35° C. and 49 days at 32° C. in females. This period is much longer in males and

varies between 31 and 75 days (mean: 57·5) at 32° C.

The development of *H. detritum* is much slower than that of *H. savignyi*. On the basis of laboratory breedings at 32° C. one generation was calculated to be completed in *c.* 146 days and we doubt whether there could be more than one complete generation a year. This corresponds also to Galuzo's (1939–41) findings.

Hyalomma marginatum

The larva. The scutum is longer than in the other species and was found to be $290 \pm 1\,\mu$ in twenty-eight specimens measured (Fig. 1*c*). The postero-external extremities of the basis capituli are smaller than in *H. savignyi*, and somewhat larger than in *H. detritum* (Fig. 2*b*). The hypostome is long and

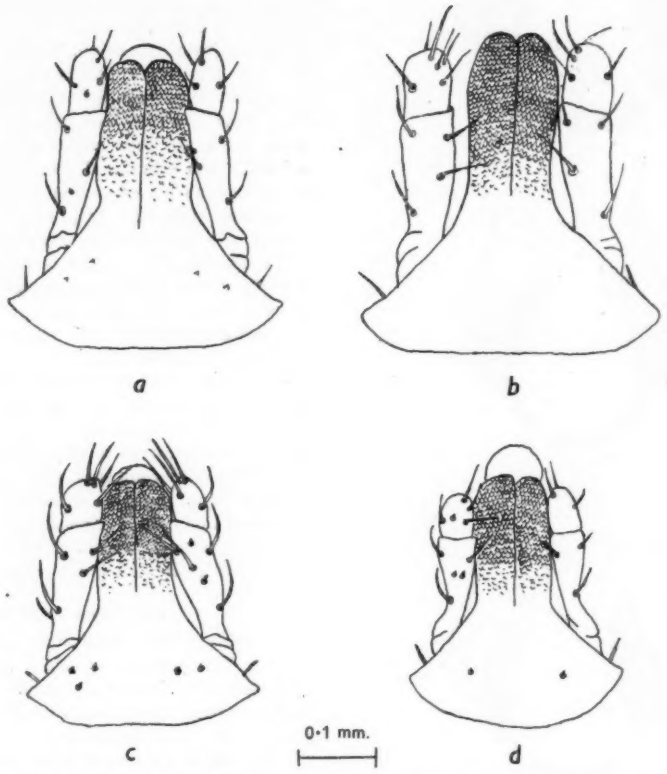

Fig. 11. Capitulum of nymphs of *Hyalomma*. (Dorsal aspect.) (*a*) *H. savignyi*; (*b*) *H. detritum*; (*c*) *H. aegyptium*; (*d*) *H. marginatum*.

The duration of the individual periods of development was as follows:

	Days
Development of egg	21
Larva (from emergence to feeding)	15
Larva (feeding)	8
Larva (from descent from host till ecdysis)	7
Nymph (from emergence to feeding)	7
Nymph (feeding)	9
Nymph (from descent from host till ecdysis)	50
Imago (from emergence to feeding)	15 (?)
Imago (feeding)	7 (?)
Imago (pre-oviposition period)	7

wide (70 μ in its widest part). It has 7–8 longitudinal rows of well-formed denticles, under which there are 1–2 rows of smaller rounded denticles (Fig. 8*c*). The spur on the first coxa is small (Fig. 3*c*). Stigmata and areae porosae dorsalis, as in *H. savignyi*.

The nymph. The nymph was studied only from the exuvia. The scutum is very short (only 490 μ) (see Fig. 12*a* and compare with that of *H. savignyi*, Fig. 12*b*). The palps are short. The hypostome is 75 μ in its widest part. The two spurs on the first coxa are moderately long (Fig. 7*d*).

Biology of H. marginatum. This species is very common on camels, but can also be found on cattle in small numbers. It was found all over Palestine from April to November.

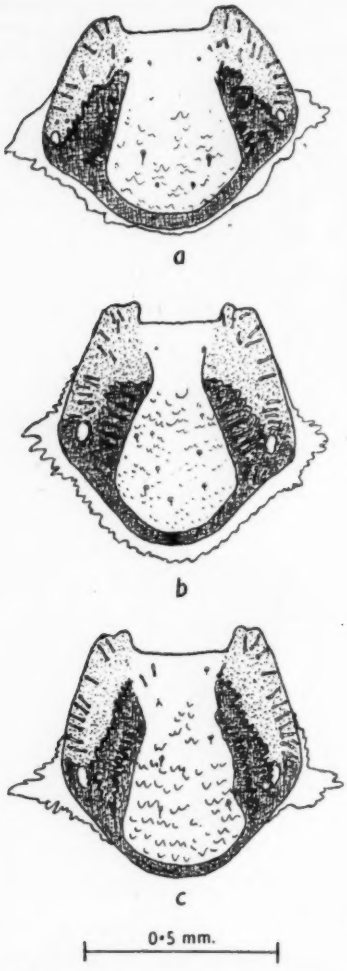

Fig. 12. Scutum of nymphs from exuviae: (*a*) *H. marginatum*; (*b*) *H. savignyi*; (*c*) *H. detritum*. The lateral extensions are seen in exuviae.

H. marginatum is very difficult to rear in the laboratory (at least on hedgehogs, rats and guinea-pigs). Nevertheless, a small number of gorged nymphs and imagines was obtained from a large number of larvae, and this enabled us to study the species.

It seems that *H. marginatum* is a 2-host tick, because no gorged larvae were ever observed to descend from the hedgehogs.

Hyalomma dromedarii

This species has been fully described by Delpy and we therefore give only a very short description, stressing only the points which are needed for comparison with the other species.

The larva. The larva is very similar to the other larvae of the genus. The scutum is relatively short $230 \pm 1\,\mu$ in two specimens (Fig. 1*b*). The postero-external extremities of the basis capituli are large (Fig. 8*b*). The hypostome is narrow ($31 \pm 7\,\mu$ in its widest part on twenty-eight specimens) and contains about four rows of well-formed denticles, under which there are four additional ranges of small, rounded, non-functional denticles. The spur of the first coxa is somewhat larger than that of *H. savignyi* (Fig. 3*b*). Stigmata and areae porosae dorsalis as in the other species of the genus.

The nymph. The nymph is so similar to that of *H. savignyi* that it is almost impossible to distinguish between them. The two spurs on the first coxa of *H. dromedarii* seems to be somewhat shorter than those of *H. savignyi* (Fig. 7*b*).

The biology of H. dromedarii. This species is very abundant on camels, and was found twice on cattle. In the south of Palestine it is also abundant during winter. The duration of one generation and that of each of the different stages are about the same as for *H. savignyi*, but the 'nymphal sleep' seems to be shorter: 14 days in *H. dromedarii* as compared with 25 in *H. savignyi*. It is therefore probable that there are several generations of *H. dromedarii* in a year.

Hyalomma aegyptium

The nymph. The colour of the nymph is dark-grey brown. The scutum is wider than long: 0·56 x 0·66 mm. The postero-external extremities of the basis capituli are prominent. The palps are short (Fig. 11*c*). The hypostome is narrow $73 \pm 0{\cdot}4\,\mu$ (twenty-eight specimens measured). The first coxa bears two short spurs (Fig. 6*c*).

The biology of H. aegyptium. This species is frequently found on tortoises; sometimes also on hedgehogs and snakes. It was found once on the little owl (*Athene noctua*).

SUMMARY

The taxonomy of larval and nymphal stages of *H. savignyi*, *H. detritum*, *H. marginatum*, *H. dromedarii* and the nymph of *H. aegyptium* are described.

The material for the first four species was obtained from laboratory-bred specimens. The bionomics of these species was studied in the laboratory. Their behaviour under natural conditions still remains to be studied.

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THE WATER RELATIONS OF THE LARVA OF *XENOPSYLLA CHEOPIS* (SIPHONAPTERA)

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CONTENTS

	PAGE		PAGE
I. Introduction	148	IV. Discussion	153
II. Methods and technique	148	V. Summary	154
III. Gain of water	149	References	155

I. INTRODUCTION

In order to maintain the normal water balance in an insect, which is essential for its usual metabolic activities, the gain of water must compensate the loss (vide Wigglesworth, 1939, p. 357). The gain of water in insects usually takes place through (1) actually drinking the liquid, (2) the cuticle, (3) the absorption of water ingested with the food, and (4) the production of water of metabolism as the result of the oxidation of food or reserve substances (*vide* Buxton, 1932, pp. 277–82). There is no evidence of the drinking habit in the flea larva, nor is it possible for it to drink water on account of the absence of free water in its environment. The gain of water through the cuticle in the flea larva may be possible by the absorption of water directly from the atmosphere and of liquid water from the soil or debris in contact with its body.

The loss of water in insects, however, is brought about (1) by evaporation through the cuticle of the general surface of the body and the tracheal system, and (2) by defaecation and excretion (*vide* Buxton, 1932, pp. 282–4). The loss of water with the contents of the alimentary canal is very great in flea larvae, as their faeces are discharged in the form of sticky semi-solid drops owing to the absence of an efficient arrangement for extracting water from them. The rectal glands, which, according to Wigglesworth (1932, p. 142), are mainly concerned in the extraction of water from faeces in many other insects, are absent in flea larvae. It is for this reason, and for a high rate of transpiration through the cuticle of flea larvae, on account of their being soil inhabitants (Wigglesworth, 1945, p. 98), that they are not very resistant to desiccation and grow only at relative humidities of 50 % and higher, especially in the case of *Xenopsylla cheopis* (Rothschild) (Sharif, 1948*a*).

According to Sikes (1931, p. 246), 'It appears that the water content of the food of flea larvae is a very important factor in breeding, and the maximum and minimum quantities probably vary for each species of flea. It is at least possible that atmospheric humidity affects the flea larva indirectly, through its food, and not only directly by regulating the loss of water from its surface.' On the other hand, Buxton (1932, p. 279) opines that Sikes, 'whose studies on the larvae of the flea *Ceratophyllus wickhami* suggest, but do not prove, that its successful development depends more on the water content of the food than that of the atmosphere'. With reference to her suggestion, subsequently Buxton (1938, p. 525) pointed out that 'This interesting point requires to be reinvestigated now that the work of Sharif (1937*a*) has supplied fuller information about the nutrition of the larvae'. In view of this controversy, it was thought desirable to find out precisely how the water is gained by the growing flea larvae. The present study is an attempt to solve this question.

II. METHODS AND TECHNIQUE

To ascertain whether the larvae of *Xenopsylla cheopis* take up water with the food, or directly from the air or both, experiments were conducted at a low effective humidity, so as to magnify the differences, if any, under the diverse experimental conditions. This requirement was satisfied by a relative humidity of 60 % in combination with a temperature of 22° C., at which almost all the larvae bred successfully on a mixed diet of blood and yeast mixed with sea sand, though there was a significant retardation in their growth (Sharif, 1948*a*) when compared with optimum humidities. In all the sets of experiments only the larvae that formed cocoons and remained within them at this humidity bred successfully owing to its low moisture content, as is mentioned elsewhere (Sharif, 1948*a*). The proportion of the cocoons spun depended upon the moisture contents of the food and of the air; a lower proportion of cocoon formation is an indication of less moisture content in the environment (Sharif, 1948*b*).

On the whole, the methods and technique adopted for conducting the experiments were similar to those described in earlier communications (Sharif, 1937*a*, p. 226; 1948*a*; 1948*b*). In these experiments completely unfed larvae less than 24 hr. old were used. They were bred in small specimen tubes that were placed in a desiccator containing an atmosphere of 60 % relative humidity, controlled by a mixture of extra pure sulphuric acid (Merck) and distilled water in a proportion given by Buxton & Mellanby (1934, p. 174). The desiccator was kept in a low-temperature incubator maintained at a temperature of 22° C. (71·6° F.) with a variation of 0·5° C. on either side.

The dry food used in the present experiments was a mixture of finely powdered dried horse blood and Merck's 'Yeast medicinal dry powder' in equal parts without sand, which was exposed to concentrated sulphuric acid for 15 days in order to drive out all the traces of moisture. In some experiments the dry food was changed either once or twice a day. In the latter case it was changed after 16 and 8 hr. alternately towards the later part of the larval life. The idea was to allow as little water as possible in the food to the larvae. In those experiments for which a large quantity of the dry food was used, each experimental tube contained 2 g. of the food. This was enough to cover the larvae completely and to allow them room to burrow, as it formed a layer of about $\frac{3}{16}$ in. thick at the bottom of a tube. The larvae burrowed deep into the food, right to the bottom of the tube, even when the conditions there were more unfavourable than those on the surface of the food. Apparently they are very strongly geotactic. In those experiments for which a small quantity of the dry food was used it was so scanty that it did not cover the larvae even if they tried to burrow in it. It was put in a very small quantity on one side of the tube, where it formed a thin layer. All these experiments were conducted under the identical conditions of temperature, humidity, darkness and pressure.

By a quantitative study of the development of the larvae of *X. cheopis*, an attempt has been made to ascertain by the aid of statistical methods if certain aspects of its biology vary when its larvae are reared under different conditions of moisture contents both in the food and in the air. For estimating the effects of these conditions on the development of the larvae the criteria adopted are (1) the duration of the complete active larval life up to the resting stage and that of the combined larval and pupal life of both the sexes, (2) mortality rate in different active larval instars and the period in which they died, (3) proportion of the resting larvae that failed to pupate, and (4) proportion of success in the rearing of adults and the disparity in their sex ratio. All these were statistically evaluated, and were subjected to appropriate tests of significance based on the mathematical expressions given by Fisher (1941) and Fisher & Yates (1943). The tests of significance tried are t and χ^2. When tested in pairs for significance, a difference which is not statistically significant is denoted by the sign of −, that which is significant at 5 % level is indicated by + and that at 1 % level by ×. The signs in each column of the tables show the level of significance between a particular value, against which a sign is placed, and the one higher in the column with which it is connected by an arrow.

III. GAIN OF WATER

In order to determine the source of gain of water by the larvae of *X. cheopis*, experiments with different moisture contents, both of the food and of the air which were in proximity to the larvae, were conducted, when the breeding tubes were exposed to a relative humidity of 60 %. This necessitated the rearing of recently hatched larvae on (*a*) a large quantity of the dry food, and (*b*) a very small quantity of the dry food, when both were changed once or twice a day; (*c*) a very small quantity of the originally dried food, and (*d*) a large quantity of the originally dried food, when both were not changed; (*e*) a large quantity of the food mixed with sea sand that was brought into equilibrium by previous exposure to this humidity. The differences in development of the larvae under the above-mentioned conditions were noted. The mixture of the food and sand, that was in equilibrium with 60 % humidity, was used for control experiments; the results obtained under other conditions were compared with those under this condition, as only precise results of the breeding of flea larvae at this humidity can be achieved under the condition of control experiments.

(*a*) *Large quantity of the dry food when changed.* All the larvae (Table 1) died within 17 days in the first instar, when kept in a large quantity of the dry food that was changed once or twice a day. As high as 70 % mortality occurred amongst them within the first 3 days. This was due to the fact that the food given to the larvae in the beginning was desiccated for 15 days and that given later on was dehydrated only for 3–5 days over concentrated sulphuric acid. Although all the larvae died without showing any appreciable signs of growth, yet they died within an average time not significantly different from that of those in a very small quantity of the dry food, whether changed or not, and from that of those in a large quantity of the dry food when not changed, even though in the last three types of experiments larvae died in different active instars. This tends to suggest that the cause of their death in all these cases was the dried food given to the larvae.

A complete failure in the rearing of the larvae (Table 1) under the conditions of this type of experiment, when compared with their almost 100 %

successful rearing in the control experiments, clearly indicates that a higher moisture content, both in the food and in the air permeating it, than that present in the former case is required for their development. The larvae had a strong tendency to burrow as deeply as possible, which deprived them even of the benefit of the entire amount of moisture present in the air on the surface of a large quantity of the dry food, as the air that filtered through the particles of the dry food lost some moisture on its way down. The dry food, and the slow penetration of the moisture of the air into it on account of the fineness of the food particles, did not permit the larvae to grow. They were thus devoid of the requisite amount of moisture both in the food and in the air close to them; consequently a significantly higher proportion of the larvae died than in all the other types of experiments which afforded them more water supply.

The fact that some of the larvae lived for more than 3 days in a large quantity of the dry food that was changed once or twice daily, shows that, on the whole, the average humidity maintained in the neighbourhood of the larvae was higher than 45 % and less than 50 %, as all the larvae of this species died within 3 days at the former humidity and a few of them bred successfully at the latter humidity (Sharif, 1948*a*). Under the present conditions the water balance of the larvae was disturbed, but not to such an extent as to kill all of them quickly; the humidity to which the larvae were subjected was fatal, but it was very near their minimal requirements. The mortality rate amongst the larvae was comparatively high when the food was changed twice a day. The larvae, especially the dead ones, were always covered with a large number of food particles and looked like small lumps of the food. The larvae shrank considerably at first and then died.

(*b*) *Small quantity of the dry food when changed.* When the larvae (Table 1) were fed on a very small quantity of the dry food, that left them exposed directly to the atmospheric humidity of 60 % throughout, and that was changed once or twice a day in order to allow as little gain of water to the larvae through the food as possible, only 42 % of them completed their active larval life. Within the first 15 days 58 % of the larvae died in different active instars, and half of these actually died in 3 days. This larval mortality was significantly higher than that in the control experiments and than that in the small or large quantity of the dry food when it was not changed, which can only be due to lack of moisture in the food in the first case. This is further borne out by the fact that a larger number of the larvae died when fed on the food dried over concentrated sulphuric acid for 15 days than on that desiccated over it for only 3–5 days. The mortality rate increased further when the dried food was changed twice daily.

The larvae developed much better comparatively (Table 1) on a very small quantity of the dry food than on a large quantity of the dry food, when both were changed, as is indicated by a significantly smaller proportion of the larvae that died in different active instars and by a significantly higher proportion of adults reared in the former case. This is due either to the difference in regulation of the loss of water at the two different humidities in the neighbourhood of the larvae, the relative humidity being less in the latter case, or perhaps to the actual gain of water through the cuticle directly from the atmospheric humidity of 60 % in the former case. On the whole, the larvae had shiny body surfaces and no food adhered to them, and they were more active when exposed directly to the air in the tubes that had a very small quantity of the dry food, than when they were covered with food particles in the tubes containing a large quantity of the dry food. This was due to the fact that direct effect of the moisture content of the air maintained at 60 % relative humidity on some of the larvae in the former case was sufficient to sustain their almost normal water balance. On account of the absence of water in the food, the larvae under the former condition can only gain water through metabolism (Sharif, 1948*a*) or probably by the absorption of water directly from the air. There is, however, a possibility of a little absorption of water by the dry food, as being in a small quantity and well spread, a greater surface area of it was exposed to the 60 % humidity for 8, 16 or 24 hr.; but this could have influenced the growth of the larvae only to a slight extent.

Only 12 % of the larvae (Tables 1, 2) were reared into adults on a very small quantity of the dry food that was changed, and their proportion was significantly less than that in the control experiments and than that in the small or large quantity of the dry food which was not changed. This is due to the fact that water balance maintained by the larvae in the first case was subnormal; though 42 % of the larvae completed their active larval life, yet the metabolic rate was so slow that a sufficient reserve food could not be stored by many of them to permit their successful metamorphic changes; consequently, 54·8 % of the larvae which completed their active larval life died in the resting stage, and their proportion was significantly higher than that in the control experiments and than that in the small or large quantity of the dry food when not changed. The complete active larval life in the first case was significantly longer than in the other three cases. All these facts tend to show that water available for the larvae in the first case was definitely less than in the other cases; for such things only occur when the water requirements of flea larvae are not completely satisfied, as is mentioned elsewhere (Sharif, 1948*a*).

Table 1.* *Showing the effects of the dry food, whether changed daily or not, and of the food in equilibrium with the humidity, on the growth of the recently hatched larvae of* Xenopsylla cheopis, *when kept at a relative humidity of 60 % in combination with a temperature of 22 ± 0·5° C.*

Quantity of food used and its state	Period for which food was changed in days: Once a day	Period for which food was changed in days: Twice a day	Experiments tried	No. of larvae used	Larvae died in different active instars: No.	Larvae died in different active instars: Sig. χ^2 test	Days in which larvae died in different active instars: Range	Days in which larvae died in different active instars: Mean and S.E.	Sig. t test	Resting larvae failed to pupate: Total resting larvae	Resting larvae failed to pupate: Resting larvae died: No.	Resting larvae failed to pupate: Resting larvae died: Sig. χ^2 test	Larvae reached the adult stage: No.	Larvae reached the adult stage: Sig. χ^2 test	No. of larvae spun cocoons	Remarks
Large, dry	9	7	2	100	100	↑	1–17	4·01 ± 0·4004	↑	0	0		0	↑	0	All larvae died in first instar
Small, dry	16	7	2	100	58	× ↑	1–15	5·19 ± 0·4928	− ↑	42	23	↑	12	× ↑	12	Two common webbings for 5 and 7 pupae
Small, dry	Not changed		1	50	13	× × ↑	2–6	3·23 ± 0·5331	− − ↑	37	7	× ↑	25	× × ↑	25	Four common webbings for 5–7 pupae
Large, dry	Not changed		1	50	8	× × − ↑	2–6	3·50 ± 0·7319	− − −	42	10	× − ↑	22	× × − ↑	22	Separate cocoons
†Large, moist	Not changed		5	173	0	× × × ×	—	—	—	173	1	× × ×	166	× × × ×	167	Separate cocoons

† Control experiments in which a mixture of food and sand, which had been brought into equilibrium with 60 % relative humidity, was used.

Table 2.* *Showing the effects of the dry food, whether changed daily or not, and of the food in equilibrium with the humidity, on the growth of the recently hatched larvae of* Xenopsylla cheopis, *when kept at a relative humidity of 60 % in combination with a temperature of 22 ± 0·5° C.*

Quantity of food used and its state	Period for which food was changed in days: Once a day	Period for which food was changed in days: Twice a day	No. of larvae completed active larval life and its duration: Larvae	Days: Range	Days: Mean and S.E.	Sig. t test	No. of females and duration of their combined larval and pupal life: ♀♀	Days: Range	Days: Mean and S.E.	Sig. t test	No. of males and duration of their combined larval and pupal life: ♂♂	Days: Range	Days: Mean and S.E.	Sig. t test	Stages died: R.L.	Stages died: E.P.	Stages died: A.P.	χ^2 for sex disproportions of adults
Large, dry	9	7	0	—	—	—	0	—	—	—	0	—	—	—	0	0	0	
Small, dry	16	7	42	16–23	18·55 ± 0·2995	↑	1	38	38·00 ± 0·0000	↑	11	39–45	41·18 ± 0·4827	↑	23	1	6	↑
Small, dry	Not changed		37	14–23	17·54 ± 0·3347	+ ↑	12	33–37	35·00 ± 0·4082	− ↑	13	38–45	40·15 ± 0·6082	− ↑	7	2	3	+ ↑
Large, dry	Not changed		42	14–20	17·33 ± 0·2328	× − ↑	7	33–36	34·57 ± 0·4286	+ − ↑	15	37–39	38·00 ± 0·1952	× × ↑	10	4	6	− − ↑
†Large, moist	Not changed		167‡	13–20	16·04 ± 0·1060	× × ×	78	28–33	30·08 ± 0·1422	× × ×	88	34–41	36·99 ± 0·1676	× × +	1	6		+ − −

* Tables 1 and 2 are continuation of each other, and experiments in the second pertain to the same batches as in the first.

† Control experiments in which a mixture of food and sand, which had been brought into equilibrium with 60 % relative humidity, was used.

‡ Only the active larvae that spun cocoons are given.

A.P. advanced pupae which contained fully formed adults; E.P. early pupae of white colour without any trace of adult formation; R.L. resting larvae ready for pupation; S.E. standard error; Sig. t test, t test of significance; Sig. χ^2 test, χ^2 test of significance; −, not significant; +, significant at 5 % level; ×, significant at 1 % level.

Even the combined larval and pupal life of both the sexes in the first case was significantly prolonged when compared with that in the control experiments and with that in the large quantity of the dry food which was not changed.

There was a marked disparity in the successful rearing of the larvae of the two sexes in a very small quantity of the dry food which was changed, as 91·7 % of the larvae (Table 2) that bred successfully were males. This disproportion between the sexes, owing to the smaller moisture content of the food was significantly different from that in the control experiments and from that in a very small quantity of the dry food that was not changed, which suggests that the water requirements of the male larvae are comparatively less than are those of the female larvae.

(*c*) *Small quantity of the dry food when not changed.* When fed on a very small quantity of the originally dried food that was not changed during the period of the experiment, 74 % of the larvae (Tables 1, 2) completed their active larval life at the atmospheric humidity of 60 %, and the rest died within the first 6 days owing to an initial deficiency of moisture in the food brought about by 15 days' exposure to the concentrated sulphuric acid. Only 50 % of the larvae were reared successfully. Of the larvae that completed their active larval life, 18·9 % died in the resting stage, and 8·1 % of them developed into adults which failed to extricate themselves from the pupal skins. Some of the resting larvae even failed to spin cocoons, though by the end of the active larval life a little more food was added to permit the defaecated larvae to form cocoons. On account of the small quantity of the substratum, i.e. the food material, four common webbings were spun by twenty-five defaecated larvae. Each webbing was a poorly formed mass of five to seven cocoons.

Development of the larvae was comparatively more successful (Tables 1, 2) on a very small quantity of the dry food which was not changed than when it was changed. This is shown by a significantly smaller proportion of the larvae that died in different active instars, by a significantly higher proportion of the reared adults and by a significantly shorter complete active larval life under the former condition than under the latter; and it definitely suggests that the larvae gained moisture from the food ingested. In the former case the originally dried food must have absorbed more moisture from the atmospheric humidity of 60 % owing to its continual exposure to this humidity for a number of days than in the latter case when the dry food was changed once or twice a day, as it is known that the food consisting of blood and yeast takes two or more days to come into equilibrium with the moisture content of the air to which it is exposed (Sharif, 1948*a*). The atmospheric humidity to which the larvae were exposed under both these conditions was the same, and only the moisture content of the food differed in the two cases. The significantly better development of the larvae in the former case than in the latter cannot be attributed to any other cause than the presence of more moisture in the food.

The fact that the average combined larval and pupal life of both the sexes (Table 2), when the larvae were bred in a small quantity of the dry food that was not changed, was not significantly different from that when they were reared in a small quantity of the dry food that was changed once or twice daily, is possibly due to small samples or perhaps to a shorter cocoon life in the latter case, which usually occurs whenever there is a slight diminution in the water normally required for flea larvae, as is mentioned elsewhere (Sharif, 1948*a*).

(*d*) *Large quantity of the dry food when not changed.* When kept in a large quantity of the originally dried food that was not changed during the period of the experiment, 84 % of the larvae (Tables 1, 2) completed their active larval life, and the remainder died within the first 6 days owing to an initial deficiency in the moisture content of the food and also of the air pervading it, especially near the bottom of the experimental tube. This is borne out by the fact that the larvae which died in the dry food that was desiccated by 3–15 days' exposure to the strong sulphuric acid, whether used in a small or large quantity and whether changed or not, died in an average time not significantly different from one another, whether they showed signs of growth or not. Of the larvae which completed their active larval life 47·6 % were not reared to adults. Of the resting larvae, 23·8 % failed to pupate, and 14·3 % of them formed adults within the pupal skins, but they could not effect emergence for want of sufficient water in their organization (Sharif, 1948*a*). The percentage of the larvae that spun individual cocoons and emerged successfully as adults was 44. The larvae were not so active when covered with a large quantity of the food as when they remained uncovered in a small quantity of the food, and the food stuck to them in large quantities in the former case.

The absence of a significant difference between the proportions of the larvae that died in different active instars and of those that were reared successfully, and between their developmental rates, when kept in a large quantity and a small quantity of the dry food, which was not changed, tends to suggest that the developing larvae in the two cases were subjected to identical conditions of moisture both in the food and in the air. Apparently, the moisture content of the air permeating a large quantity of the dry food came into equilibrium with that on the surface of the food within a few days, and thus, on the whole, it did not influence the growth of the larvae differently from that in the atmospheric humidity of the

tube containing a small quantity of the dry food that was not changed. There was, however, a significant reduction in the average combined larval and pupal life of the male under the former condition; though there was no significant difference between the disproportions of the sexes in the two cases. This is possibly due to the lesser water requirements of the male larvae, as has been suggested before (see p. 152). As the result of an observation based on rearing several thousands of the larvae of this species under favourable conditions of humidity, it was found that the female larvae, as a rule, spin cocoons and pupate earlier than do the male larvae (Sharif, 1948*a*). Thus, perhaps on account of a longer contact with the large quantity of the food in equilibrium with the low effective humidity of 60 %, the male larvae, with their smaller water requirements, developed comparatively quicker and in a larger proportion than did female larvae; or perhaps the early effects on the female larvae of the initial deficiency of moisture in a large quantity of the dry food and in the air pervading it, resulted in their comparatively slower growth rate. The former alternative seems to be more probable and suggests that flea larvae can absorb moisture from the food in contact with them.

(*c*) *Large quantity of the food and sand mixture in equilibrium with the humidity.* In the control experiments, for which a mixture of 1 g. of the food and 5 g. of ignited and acid-washed sea sand, which had been brought into equilibrium with a relative humidity of 60 % by previous exposures of 2–8 days to it, was used, 100 % of the larvae (Tables 1, 2) completed their active larval life, 96·5 % of them spun individual cocoons, and 96 % of them emerged successfully. The proportion of the emerged adults was significantly higher, and their average combined larval and pupal life was significantly shorter, in the control experiments than when they were reared in a large quantity of the originally dried food that was not changed, or in a very small quantity of the dry food whether changed or not. The complete active larval life up to cocoon formation was significantly shortened, and the proportion of the larvae that died in different active instars was significantly the least in the first case. Evidently, under the present conditions, the moisture content of the food and that of the air permeating the food and sand mixture were enough to give about 100 % successful rearing of the larvae, and thus satisfied all the necessary requirements of a control experiment.

IV. DISCUSSION

By taking advantage of delay in the absorption of water from the air by the dried food comprising blood and yeast, it has been shown experimentally that flea larvae gain water through the imbibition of water taken with the food, and this appears to be the main source of water gain. This fact is strongly supported by the observation of Sikes (1931, p. 245), who found that diverse diets given to the larvae of *Orchopeas wickhami* (Baker) had different hygroscopic powers, and that the optimal atmospheric humidity for their rearing differed according to the kind of food employed, so as to supply them with optimal water content. Her observation gains further support from my experiments on the effects of different diets on the development of the larvae of the three Indian rat-fleas belonging to the genus *Xenopsylla* Glinkiewicz (Sharif, 1948*b*). The failure to rear larvae of these fleas on blood alone was due to the fact that dried horse blood, in addition to its low nutritive value, is incapable of absorbing enough moisture, even from an atmospheric humidity of 80 %; but the addition of dried medicinal yeast to it increased the hygroscopic property of this mixed larval food, which then allowed the growth of the larvae even at lower relative humidities of 50 and 60 % (Sharif, 1948*a*).

The next question is whether flea larvae gain moisture directly from the air and from the soil in their neighbourhood or whether the atmospheric humidity acts simply by regulating the loss of water from their body surface according to its drying power. It is, however, suggested that insects like flea larvae, whose equilibrium humidity, that is, the humidity at which the rate of evaporation is reduced to such a level that the loss of water alone does not result in their death, is 50 % or higher, might gain water directly from the air, or from the soil in the case of soil-inhabiting insects, when kept at humidities favourable for their development. There is no experimental evidence in support of such a presumption; but there is indirect evidence which tends to show that such a thing occurs in nature. It has been observed that certain starved insects (see for instance, Buxton, 1932, pp. 280–2; Wigglesworth, 1939, pp. 356–7; Govaerts & Leclercq, 1946, p. 483) and starved ticks (Lees, 1946, pp. 6, 7) absorb moisture directly from the atmosphere, especially if it is saturated or nearly saturated, and that the site of uptake of water is the general surface cuticle. It is suggested that flea larvae, when bred at a low effective humidity of 60 %, were in a physiological state of semi-starvation, as a greater part of the food ingested was utilized for the production of metabolic water (vide Sharif, 1948*a*), and that they might be actually gaining water directly from the air, as is indicated by the development of some of the larvae, when they were fed on a very small quantity of the dried food which was changed once or twice a day and were directly exposed to this low humidity (see p. 150). There is, however, a little experimental evidence in favour of the absorption of water directly from the substratum in which the larvae lived (see

p. 153), as has been described by Evans (1943, p. 21) in wireworm larvae. Lees has actually demonstrated the gain of water through the cuticle in the unfed female adults and engorged nymphs of ticks by keeping them between moistened pads of filter paper.

It is well known that insects both gain and lose water through the cuticle. According to Lees (1946, p. 17), the exchange of water in ticks occurs mostly through the cuticle and the tracheal system appears to play 'relatively little part in the exchanges'. The fact that the larvae of *X. cheopis* grow at 50 % relative humidity in combination with the medial temperatures, and fail to grow, even at 60 % relative humidity, at the extreme temperatures (Sharif, 1948*a*), suggests that they exercise some control on the loss of water in the former case, possibly by the spiracular closing mechanism, which is not possible at extreme temperatures, as the mechanism is thrown out of action. Apparently, in the former case the loss of water mostly takes place through the surface cuticle; whilst in the latter case water is lost through both the tracheal system and the surface cuticle. A greater loss of water in the latter case cannot be explained otherwise than by the spiracles being held permanently open, as it is well known that the loss of water in insects is many times more when the spiracles are caused to open by exposing them to carbon dioxide (see for instance, Mellanby, 1934, pp. 142–8; Wigglesworth, 1935, pp. 404–6). The presence of a very simple type of internal closing apparatus has been described by me (Sharif, 1937*b*, p. 508) in the abdominal spiracles of the larva of *Nosopsyllus fasciatus* (Bosc). The tracheal valve is a semicircular pouch-like inpushing of the front wall of the atrium, and it is controlled by an occlusor muscle. A similar closing apparatus may be present in the larva of *Xenopsylla cheopis*; but, as is pointed out by Buxton (1938, p. 524), it is less efficient than in the adult; and consequently, the loss of water through the tracheal system of the flea larva is much greater than in the adult.

According to Lees (1946, p. 10), the regulation of the water balance in ticks is solely controlled by the activity of the epidermal cells. The ability to retain water is often attributed to the physical properties of a film of lipoid overlying the epicuticle, which in most soil-inhabiting insects such as flea larvae is often abraded; consequently, the loss of water by evaporation and uptake of water through their cuticle can easily occur (Wigglesworth, 1945, p. 106). Possibly the regulation of water balance in the flea larva can also be achieved by the secretory activity of the epidermal cells as in ticks.

Hurst (1941, p. 389), however, has suggested that the asymmetrical structure of the insect cuticle has some relationship with differences in the rates of flow across it. He found that in the isolated blowfly cuticle the rate of diffusion from epicuticle to endocuticle was more than one hundred times greater than in the opposite direction. Similarly, Lees (1946, pp. 16, 19) found that the ticks *Rhipicephalus sanguineus* (Latreille) and *Dermacentor andersoni* Stiles gained water in saturated air up to three and ten times respectively as rapidly as they lost it in dry air. Both the evaporation and uptake of water through the cuticle of a living arthropod, as is pointed out by Lees, is a complex phenomenon, and cannot be defined adequately in terms of evaporation or uptake of water from a simple membrane. In this connexion we 'have to take into consideration the physiological state and functional abilities of the organism'. The uptake of water through the cuticle can only be possible at humidities higher than the equilibrium humidity which varies in different insects; for the larvae of *Xenopsylla cheopis* it is between 50 and 60 % humidities, as when unfed they died at 50 % relative humidity in a significantly shorter time than at 60 % humidity (Sharif, 1948*a*). The gain of water through the insect cuticle at high humidities has been demonstrated in many insects; but it has never been done at low effective humidities. Theoretically, it should be possible in the latter case as well, especially when the insects are badly in need of water from every available source; but its demonstration would be very difficult. The fact that the growth rate of flea larvae is not controlled by any particular value of saturation deficiency (Sharif, 1948*a*) suggests that they gain water through the cuticle. It is, however, suggested that the gain of water from the air, or from the soil in contact with the cuticle, may be a supplementary source of water for flea larvae, especially as they can only grow at relative humidities higher than 50 %.

V. SUMMARY

A considerable loss of water in the flea larva, through its defaecation and excretion as the result of the absence of rectal glands, through a profuse evaporation from the surface cuticle on account of its being a soil inhabitant, and from its tracheal system owing to the absence of an efficient closing apparatus of the spiracles, is compensated by the absorption of water with the food and through the cuticle, and by the utilization of metabolic water.

It has been proved, by conducting experiments on the food composed of dried horse blood and yeast, with different degrees of moisture contents, and by controlling the quantity of the desiccated food and the duration of its exposure to a low effective humidity of 60 % at 22° C., that the chief source of the gain of water in the larva of *Xenopsylla cheopis* is through the food.

The closing mechanism of the spiracles of the flea larva plays only a small part in the control of

evaporation at medial temperatures but not at extreme ones.

There are intrinsic differences in the water requirements of the flea larvae of the two sexes, being slightly less in the male larva than in the female larva.

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